Journal of Controlled Release 149 (2011) 21–28

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Polyamidoamine dendrimers as novel potential absorption enhancers for improving the small intestinal absorption of poorly absorbable drugs in rats

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ARTICLE INFO

Article history:
Received 14 October 2009
Accepted 12 February 2010
Available online 22 February 2010

Keywords:
Intestinal absorption
Polyamidoamine (PAMAM) dendrimer
Absorption enhancer
Drug absorption
Absorption enhancement
Intestinal toxicity

ABSTRACT

Effects of polyamidoamine (PAMAM) dendrimers on the intestinal absorption of poorly absorbable drugs were examined by an *in situ* closed loop method in rats. 5(6)-Carboxyfluorescein (CF), fluorescein isothiocyanate-dextrans (FDs) with various molecular weights, calcitonin and insulin were used as model drugs of poorly absorbable drugs. The absorption of CF, FD4 and calcitonin from the rat small intestine was significantly enhanced in the presence of PAMAM dendrimers. The absorption-enhancing effects of PAMAM dendrimers for improving the small intestinal absorption of CF were concentration and generation dependent and a maximal absorption-enhancing effect was observed in the presence of 0.5% (w/v) G2 PAMAM dendrimer. However, G2 PAMAM dendrimer had almost no absorption-enhancing effect on the small intestinal absorption of macromolecular drugs including FD10 and insulin. Overall, the absorption-enhancing effects of G2 PAMAM dendrimer in the small intestine decreased as the molecular weights of drug increased. However, G2 PAMAM dendrimer did not enhance the intestinal absorption of these drugs with different molecular weights in the large intestine. Furthermore, we evaluated the intestinal membrane damage with or without G2 PAMAM dendrimer. G2 PAMAM dendrimer (0.5% (w/v)) significantly increased the activities of lactate dehydrogenase (LDH) and the amounts of protein released from the intestinal membranes, but the activities and amounts of these toxic markers were less than those in the presence of 3% Triton X-100 used as a positive control. Moreover, G2 PAMAM dendrimer at concentrations of 0.05% (w/v) and 0.1% (w/v) did not increase the activities and amounts of these toxic markers. These findings suggested that PAMAM dendrimers at lower concentrations might be potential and safe absorption enhancers for improving absorption of poorly absorbable drugs from the small intestine.

1. Introduction

Oral administration offers several advantages over parenteral drug administration such as the elimination of pain and discomfort associated with injections, and lower costs to produce oral formulations. Therefore, many efforts have been made to develop the oral drug delivery systems to enhance the intestinal absorption of drugs with poor bioavailability. However, it is well known that the intestinal absorption of hydrophilic and macromolecular drugs including peptide and protein drugs is typically very poor [1,2]. The low bioavailability of these drugs is mainly due to their extensive degradation in the gastrointestinal tract by various peptidase and digestive enzymes and their low membrane permeability characteristics [1,2]. Therefore, various strategies including the use of additives including absorption enhancers [3–11], protease inhibitors [12–15], chemical modification of drugs with some moiety [16–23] and development of new dosage forms [24–27] have been examined to improve the intestinal absorption of these drugs. Among these strategies, absorption enhancers including surfactants, bile salts, chelating agents and fatty acids have been used to enhance the bioavailability of these poorly absorbable drugs [3]. However, the absorption-enhancing effects of many conventional absorption enhancers were generally greater in the large intestine than those in the small intestine [6,8,11], although the small intestine has anatomically large surface area as compared with the large intestine, which was advantageous for drug absorption into the systemic circulation. Therefore, it is desired to develop a novel absorption enhancer which is much more effective in the small intestine rather than in the large intestine.

Polyamidoamine (PAMAM) dendrimers are the first complete dendrimers family to be synthesized, characterized and commercialized [28,29]. They show an exciting new class of macromolecular architecture called dense star polymers. They have a unique tree-like amidoamine repeat branching architecture that starts from an initiator core of ethylene diamine. Each consecutive series of the branching step is termed a generation (G). Full generation PAMAM dendrimers are

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doi:10.1016/j.jconrel.2010.02.017

terminated with amine surface groups (G0, G1, G2, G3, etc.), whereas half generation dendrimers are terminated with carboxylate (G1.5, G2.5, etc.). PAMAM dendrimers can exist as positively charged amine-terminated, neutral hydroxyl-terminated or negatively charged carboxyl-terminated. Unlike classical polymers, dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution, specific size, shape characteristics and a highly-functionalized terminal surface [29].

Recently, PAMAM dendrimers have shown the greatest potential for drug delivery [30–36]. Namely, PAMAM dendrimers have been applied in several pharmaceutical and drug delivery fields such as improving the solubility of poorly water-soluble drugs, controlling drug release, improving the gene delivery as drug carriers. In addition, it was found that some of these dendrimers could be taken up into the intestinal epithelial cells by an endocytosis mechanism [37,38]. Although many researchers studied the effects of PAMAM dendrimers on the pulmonary and transdermal absorption of drugs and transport mechanisms of PAMAM dendrimers themselves [28–30,35,37], few studies have been examined to investigate the absorption-enhancing effects of PAMAM dendrimers as novel absorption enhancers for improving the intestinal absorption of poorly absorbable drugs in rats.

In this study, therefore, 5(6)-carboxyfluorescein (CF), fluorescein isothiocyanate-dextrans (FDs) with various molecular weights, calcitonin and insulin were chosen as models of poorly absorbable drugs and we examined the effects of PAMAM dendrimers on the intestinal absorption of these poorly absorbable drugs with the different molecular weights in rats. In addition, the intestinal membrane damage of PAMAM dendrimers was also evaluated by measuring the activities of lactate dehydrogenase (LDH) and the amounts of protein released from the intestinal membranes in rats.

2. Materials and methods

2.1. Materials

CF was obtained from Eastman Kodak Co. (Rochester, NY, USA). PAMAM dendrimers (Generation 0, 1, 2, 3), FD with an average molecular weight of 4400 (FD4) and FD with an average molecular weight of 9100 (FD10) were supplied from Sigma-Aldrich Chemical Co. (St. Louis, MO). Insulin was obtained from Nacalai Tesque (Kyoto, Japan). Calcitonin acetate (salmon) and LDH CII assay kit were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bicinchoninic acid (BCA) protein assay kit was provided from Pierce Biotechnology Inc. (Rockford, USA). All other reagents were of analytical grade.

2.2. Preparation of drug solutions

CF, FD4, FD10, calcitonin and insulin were dissolved in Hepes–Tris buffer solution at pH 7.4 to yield a final concentration of 0.04 mg/ml, 0.64 mg/ml, 0.64 mg/ml, 6.4 μg/ml, and 6.4 IU/ml, respectively.

In our study, methanol solutions of PAMAM dendrimers were evaporated at 60 °C up to the constant weight and the resulting residues were dissolved in water. A series of concentration (w/v) of PAMAM dendrimers were added to the drug solution as described above for absorption studies.

2.3. Intestinal absorption of drugs by an in situ closed loop method

Intestinal absorption of drugs was examined by an *in situ* closed loop method, as reported previously [12]. Male Wistar rats (250–300 g) were fasted overnight and anesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). The experiments were carried out in accordance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. Rats were fasted for approximately 16 h before the experiment but water was freely available. Rats were placed under a heating lamp to maintain the body temperature around 37 °C. The intestine was exposed through the midline abdominal incision. After ligating the bile duct, the small intestine or the large intestine was washed with Tris–Hepes buffer solution (25 mM Hepes, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, pH 7.4 by 1 M Tris). The remaining buffer solution was expelled with air. The small or large intestine was cannulated at both ends using polyethylene tubing, and the distal part of the small or large intestine was closed by clipping a forceps. Drug solutions with or without PAMAM dendrimers, kept at 37 °C were introduced directly into the lumen of the intestinal loop through a cannulated opening in the proximal part of the small or large intestinal loop, which was then closed by clipping with another forceps. The jugular vein was exposed and blood samples (~0.3 ml) were collected into heparinized syringe at predetermined time intervals up to 240 min. Samples were immediately centrifuged at 10,000 rpm for 5 min to obtain the plasma fraction, which was then kept in ice until determination. The drug concentrations in these plasma samples were determined by the methods as described below.

In the case of pretreatment experiment, G2 PAMAM dendrimer (0.5% (w/v)) was introduced into the small intestine loop. The dendrimer solution was removed by washing the small intestine with buffer solution after pretreatment for 10, 60 and 120 min, respectively. After washing the small intestine with buffer solution, CF solution was administered into the small intestinal loop as indicated above and then collected the blood samples for determination of CF concentrations.

The peak concentrations (Cmax) and the time to reach the peak concentrations (Tmax) were determined directly from the plasma concentration–time profiles. The area under the curve (AUC) was calculated by the trapezoidal method from time zero to the final sampling. The absorption enhancement ratios of drugs with or without PAMAM dendrimers were calculated as follows.

$$\text{Absorption enhancement ratio} = AUC_{\text{with enhancer}} / AUC_{\text{control (without enhancer)}}$$

2.4. Assessment of membrane damage

To evaluate small intestinal membrane damage in the presence of PAMAM dendrimer, the activities of LDH and the amounts of protein released from the small intestinal membranes were measured by an *in situ* loop method, as reported previously [39,40]. G2 PAMAM dendrimer (0.05–0.5% (w/v)) or Triton X-100 (as a positive control) was administered into the small intestinal loop in a similar manner to that used for the absorption experiments. Rats were left for 4 h after administration and at the end of the experiments, the perfusate in the small intestinal lumen was washed with Tris–Hepes solution for the determination of the activities of LDH and the amounts of protein released from the small intestinal membranes. The activities of LDH were determined using LDH CII assay kit (Wako Pure Chemical Industries, Ltd.). The concentrations of protein were measured with bovine serum albumin as a standard using a BCA protein assay kit (Pierce Biotechnology Inc.).

2.5. Analytical methods

The fluorescence intensities of CF, FD4 and FD10 in plasma were determined with a fluorescence spectrophotometer (Spectrafluor Plus, Tecan) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm after pretreatment the plasma with the same volume of acetonitrile.

Plasma calcium was measured by Calcium E Test kit (Wako Pure Chemical Industries, Osaka, Japan) [41,42]. Plasma insulin was determined by Insulin–EIA TEST (Wako Pure Chemical Industries, Osaka, Japan), while plasma glucose level was measured by a glucose oxidase method (Glucose B Test Wako Kit, Wako Pure Chemical Industries, Osaka, Japan).