Drug Development and Industrial Pharmacy, 2010; 36(9): 1027–1035

informa
healthcare

ORIGINAL ARTICLE

Preparation and characterization of puerarin–dendrimer complexes as an ocular drug delivery system

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Abstract

Objective: The aim of this study was to prepare and characterize the complex of puerarin and poly(amidoamine) (PAMAM) dendrimers and to evaluate the complex as an ocular drug delivery system. *Methods*: The complexes of puerarin and PAMAM dendrimers were prepared at various puerarin-to-dendrimer ratios. The physicochemical properties of the complexes were characterized by differential scanning calorimetry and Fourier transform infrared spectroscopy. The in vitro release studies were performed by dialysis. Corneal permeation was evaluated by Valia-Chien diffusion cell with excised corneas and ocular residence time in rabbits. *Results*: The results showed that puerarin-dendrimer complexes formed primarily by hydrogen-bonding interactions. Typically, 43, 56, 125, and 170 molecules of puerarin could be incorporated into G3.5, G4, G4.5, and G5 PAMAM dendrimer molecule. Puerarin was released more slowly from puerarin-dendrimer complexes than free puerarin in deionized water and phosphate buffer solution (pH 6.8). The in vitro release rate of puerarin complexed with full generation dendrimers was lower than that with half generation dendrimers. Furthermore, puerarin-dendrimer complexes produced longer ocular residence times in rabbits compared with puerarin eye drops. No damages to the epithelium or endothelium were observed after the PAMAM dendrimer administration in this corneal permeation study. *Conclusions*: Puerarin-dendrimer complexes represent a potential ocular drug delivery system to improve the efficacy of drug treatment.

Key words: *Complex; in vitro release; ocular drug delivery system; PAMAM dendrimer; puerarin*

Introduction

Puerarin (aqueous solubility, 3 mg/mL) is an isoflavone compound extracted from the radix of *Pueraria lobata* (Willd.) Ohwi[1,2]. It is frequently used as a therapeutic agent for cataracta glauca and ocular hypertension[3] because of its ability to depress intraocular pressure and to improve ocular blood flow. A current clinical product is puerarin eye drops (1%, w/v). Various strategies have been used to improve the solubility of puerarin, including adding cosolvents, surfactants, and cyclodextrin[4,5]. However, adding high concentrations of these agents may cause some adverse effects because of high viscosity and osmosis. Puerarin eye drops typically drain from the ocular surface rapidly, which results in short residence time, short absorption time (only a few minutes), and low bioavailability (less than 5%)[6]. Therefore, a new drug delivery vehicle that enhances the solubility of puerarin and prolongs cornea exposure time is urgently needed.

The application of synthetic polymers has been recognized as a potential approach for the development of novel drug carriers. Dendrimers, reported for the first time in the early 1980s[7], are three-dimensional, tree-like nanopolymeric particles with branches emanating from a central core. The precise control of size, shape, and end-group functionality can be achieved through step-by-step synthesis, using divergent and convergent approaches[7–9]. The empty internal cavities and open conformations of dendrimers make it possible to encapsulate hydrophobic drug molecules and thus increase their aqueous solubility[10,11]. In addition, dendrimers possess a high density of surface functional groups, thus allowing them to be modified or conjugated

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(Received 10 Oct 2009; accepted 11 Jan 2010)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639041003610799

with a variety of guest molecules[12–14] to facilitate multivalent interactions with biological membranes and drugs[15]. For example, cationic dendrimers are known to interact with negatively charged biological membranes to increase their permeability[16–18]. The cornea is negatively charged at physiological pH; therefore, this unique feature could be an advantage for an ocular drug delivery system[19]. In support of this idea, Vandamme and Brobeck[20] have demonstrated that poly(amidoamine) (PAMAM) dendrimers can increase the corneal residence time of pilocarpine nitrate and tropicamide, and there were very few studies about PAMAM dendrimers on ocular drug delivery system. Therefore, the development of a novel ocular drug delivery using dendrimers may be a promising approach for clinical applications.

In this study, puerarin–dendrimer complexes were prepared and their physicochemical properties, in vitro release, corneal permeation, and ocular residence times were determined.

Materials and methods

Materials

PAMAM dendrimers with primary amine (G4, G5) and carboxylate (G3.5, G4.5) surface groups in methyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Puerarin was purchased from Shanghai DND Pharm-Technology Co. (Shanghai, China). Oxidized glutathione was purchased from Beijing Yili Fine Chemicals Co. Ltd. (Beijing, China). All other chemicals and solvents were purchased from Shanghai Chemical Co. (Shanghai, China).

New Zealand albino rabbits (2.5–3.0 kg) were obtained from the experimental animal center of Luye Pharma (Yantai, Shandong, China). The animals were housed in standard cages in a light-controlled room at 19 ± 1°C and 50 ± 5% relative humidity separately and given a standard pellet diet and water. All animals were healthy and free of clinically observable ocular abnormalities. All studies were conducted in accordance with the principles of Laboratory Animal Care (NIH publication no. 92–93, revised in 1985) and were approved by the local ethics committees for animal experimentation.

Preparation of puerarin–dendrimer complexes

Dendrimers were added to the puerarin methanol solution at a dendrimer-to-puerarin ratio of 1:2.5, 1:3, 1:3.5, 1:5, 1:10 (w/w) and stirred for 24 hours in dark, followed by methanol removal by rotating evaporator at room temperature[21]. The precipitates were vacuum-dried to completely remove residual methanol. Deionized water was added and the solutions were stirred in dark for 12 hours to extract the puerarin–dendrimer complexes and remove free puerarin.

The solutions were centrifuged at 6236×*g*/min for 5 minutes and then filtered through 0.45-μm hydrophilic polytetrafluoroethylene (PTFE) membranes (45 mm diameter; Omnipore, Millipore, MA, USA)[22]. Lyophilization was performed to obtain puerarin–dendrimer complex powder. Lyophilization processes are as follows: the samples were firstly frozen at −25°C for 30 minutes, then the frozen products were placed in a vacuum and gradually heated from −25°C to 24°C in 14 hours to make ice sublimation, and then maintained at 24°C for 6 hours to achieve the lyophilized products.

Ultraviolet spectroscopy

Puerarin in distilled water gives maximum absorbance in ultraviolet (UV) region at its characteristic wavelength (249 nm for puerarin). However, the dendrimers give weak absorbance at this wavelength. As the dendrimers give on absorbance in UV region at 255–275 nm, we choose 260 nm where puerarin gives more absorbance than dendrimers, the absorbance obtained from puerarin–dendrimer complex would be solely from puerarin. An UV spectrometer (Shimadzu Co.; Shimadzu, Japan) was used to estimate the amount of puerarin incorporated in the dendrimer (λ_{max} = 260 nm). The calibration curve of puerarin solution was linear over a concentration range of 2–12 μg/mL (r^2 = 0.9999). The absorbance of puerarin at λ_{max} = 260 nm was related with the calibration curve and the amount of puerarin in puerarin–dendrimer complex was determined[23]. As the amount of dendrimer was known, we expressed the results as the number of puerarin molecules incorporated per dendrimer molecule.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR spectroscopy, Perkin Elmer-Spectrum One, Waltham, MA, USA) was used to study the interaction between puerarin and dendrimers[24,25]. The IR spectra of free puerarin, dendrimers, and puerarin–dendrimer complexes were obtained by the potassium bromide (KBr) pellet method.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were conducted using a DSC-822 system (METTLER-TOLEDO Greifensee, Switzerland). Samples containing 0.5 mg of puerarin alone or puerarin–dendrimer complexes were heated at a scanning rate of 10 K/min from 25°C to 250°C in hermetically sealed aluminum pans under nitrogen purge.

In vitro release studies

In vitro release of free puerarin and puerarin–dendrimer complexes was performed in deionized water, phosphate buffer solution (PBS) (pH 6.8), and hydrochloric acid solution (pH 1.5) by dialysis. Puerarin and puerarin–dendrimer complexes were dissolved in solution at a concentration equivalent to 2 mg/mL puerarin. These solutions (1 mL) were transferred into dialysis bags (molecular weight cutoff = 3500 Da) and dialyzed against 20 mL solution at 25°C. This molecular weight cutoff was used because the dendrimers have a significantly higher molecular weight, so that they could stay inside the dialysis membrane, whereas the small molecular weight drug would readily diffuse out of the dialysis bag. The outer phase was stirred continuously. Samples (1 mL) were withdrawn from the receiver solution at 0.25, 0.5, 0.75, 1, 1.5, 3, 6, and 9 hours, followed by the addition of an equal volume of solution to maintain a constant volume. Puerarin in the receiver solution was determined at various time points by UV spectroscopy.

Corneal permeation studies

The corneal permeation studies were performed using a Valia-Chien diffusion cell (Kimble Bomex glass Co. Ltd., Beijing, China) with donor and reservoir chambers. The corneas, excised with a 2-mm ring of sclera, were mounted in the Valia-Chien diffusion cell at 37°C with an available area of 0.785 cm^2. The donor cell and the reservoir cell were filled with a sample solution (4.5 mL puerarin or puerarin–dendrimer complex) and glutathione bicarbonate ringer (GBR) buffer (5 mL), respectively, with an O_2 : CO_2 (95:5) mixture bubbling through each compartment. A 200-μL sample was withdrawn from the reservoir cell at regular time intervals up to 2.5 hours, followed by an addition of an equal volume of glutathione bicarbonate ringer to maintain a constant volume. Puerarin concentrations in the reservoir cell were determined by high-performance liquid chromatography at various time points including 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5 hours, and so on.

The apparent corneal permeability coefficient (P_{app}; cm/h) was measured using the following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t \times C_0 \times A},$$

where $\Delta Q/\Delta t$ is the steady-state slope of the linear portion of the plot for the amount of drug permeated (Q) versus time (t). A is the exposed cornea surface (0.785 cm^2) and C_0 is the initial concentration of drug in the donor cell.

After corneal permeation, each tested cornea was carefully removed from the scleral ring and weighed. The wet corneal weights (W_w) and dry corneal weights (W_d, after desiccation at 70°C for 6 hours) were used to determine the corneal hydration level (HL%; defined as $[1–(W_d/W_w)] \times 100$), compared with the weights of untreated corneas (removed no longer than 30 minutes after the death of the animal).

Determination of ocular residence time

Puerarin–dendrimer complex solutions (50 μL) and puerarin eye drops (50 μL) were instilled onto the center of the right and left corneas of each male New Zealand albino rabbit, respectively (n = 7); the eyelids were kept closed for 10 seconds to prevent loss of the instilled solution. A filter paper slip (5 × 2 mm) was placed into the conjunctival fornix to collect tears from each eye at 0.25, 0.5, 0.75, 1, 2, 4, and 6 hours for the determination of puerarin by high-performance liquid chromatography.

Data analysis

Results are reported as the mean ± SD. Student's t-test was used to identify differences that were considered to be statistically significant at $P < 0.05$ and $P < 0.01$.

Results

Characteristics of puerarin–dendrimer complexes

As shown in Figure 1, the puerarin-to-dendrimer molar ratios were 43:1 (G3.5), 56:1 (G4), 125:1 (G4.5), and 170:1 (G5). These results suggest that the amount of incorporated puerarin increased with the increase of the dendrimer size (both $–NH_2$ and $–COOCH_3$ terminated).

Next, the effects of various pH conditions on the preparation of puerarin–dendrimer complexes were

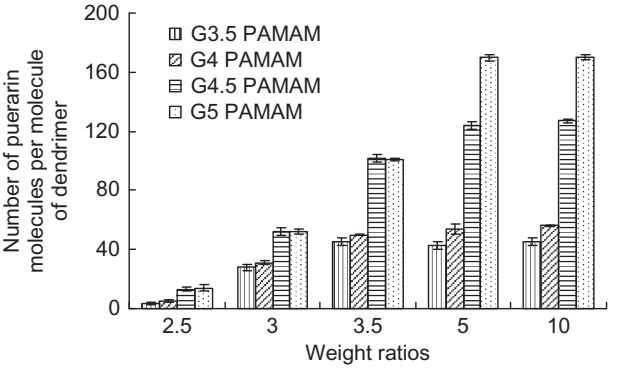

Figure 1. Effect of puerarin-to-dendrimer weight ratio on puerarin incorporation into PAMAM dendrimers. The puerarin-to-dendrimer weight ratios were 2.5:1, 3:1, 3.5:1, 5:1, and 10:1. Maximum number of puerarin molecules incorporated was 170 per molecule G5 PAMAM dendrimer. Data are expressed as mean ± SD from three independent experiments.

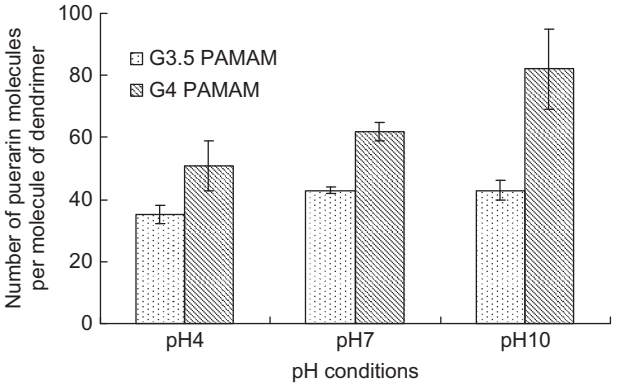

Figure 2. Effect of pH on puerarin incorporation into PAMAM dendrimers. The ratio of puerarin-to-dendrimer was determined under different pH conditions. In the G4 PAMAM puerarin–dendrimer complex, the ratio increased from 45 to 80 with increasing pH. In contrast, the ratio of puerarin-to-dendrimer in the G3.5 PAMAM puerarin–dendrimer complex increased only slightly, from 38 to 40. Data are expressed as mean ± SD from three independent experiments.

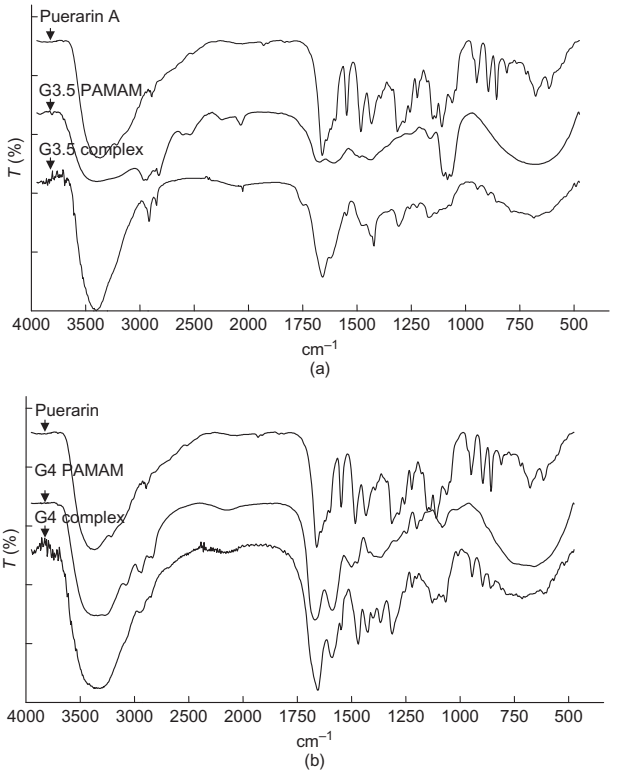

Figure 3. Comparative FTIR spectrum of puerarin, PAMAM dendrimers, and puerarin–dendrimer complexes: (a) puerarin, G3.5 PAMAM dendrimer, and G3.5 PAMAM puerarin–dendrimer complex; (b) puerarin, G4 PAMAM dendrimer, and G4 PAMAM puerarin–dendrimer complex.

determined. As shown in Figure 2, higher pH increased the puerarin-to-dendrimer ratio of the G4 PAMAM puerarin–dendrimer complex; however, pH had little effect on the ratio in G3.5 PAMAM puerarin–dendrimer complexes.

FTIR spectroscopy (4000–400 cm^{-1}) was used to analyze puerarin, various PAMAM dendrimers (G3.5 and G4), and puerarin–dendrimer complexes. These FTIR spectra were shown in Figure 3. Pure puerarin demonstrated a strong absorbance at 3396 cm^{-1}, which corresponds to the hydroxyl group (OH). Other peaks in the regions 1600–1500 cm^{-1} and 1200–1000 cm^{-1} were attributed to the benzene ring[26]. These typical absorption bands of puerarin were covered and/or overlapped by pure G3.5 PAMAM dendrimer in the G3.5 PAMAM puerarin–dendrimer complex spectrum. Shifts to lower frequencies were observed in the C = O stretching of the ester group (from 1398 to 1385 cm^{-1}) (symmetric) and the amide I band (C = O stretching from 1645 to 1631 cm^{-1}) of the G3.5 PAMAM dendrimer. A similar spectrum pattern was observed for the G4 PAMAM puerarin–dendrimer complex. The FTIR spectrum for the G4 PAMAM puerarin–dendrimer complex showed the characteristic absorption bands of G4 PAMAM dendrimer and puerarin. The O–H absorption bands of puerarin shifted to lower frequencies from 3396 to 3350 cm^{-1} and the N–H absorption bands of G4 PAMAM dendrimer shifted from 3366 to 3350 cm^{-1}.

DSC thermograms of puerarin and puerarin–dendrimer complex are shown in Figure 4. The dendrimers did not produce peaks in the temperature range of 25–250°C, whereas puerarin produced a sharp endothermic melting peak (T_m) at 190.3°C. However, this peak disappeared in G3.5 and G4 PAMAM puerarin–dendrimer complexes, which indicated that puerarin binds with G3.5 and G4 PAMAM dendrimers[26,27].

In vitro release studies

In vitro release of puerarin from puerarin–dendrimer complexes was investigated in three solutions: deionized water, PBS (pH 6.8), and hydrochloric acid solution (pH 1.5) (Figure 5). The puerarin–dendrimer complexes produced different release profiles in these three solutions. Overall, the complexes demonstrated the most rapid release rate in hydrochloric acid solution (pH 1.5), whereas the complexes demonstrated the slowest release rate in deionized water. Compared to free puerarin, the complexes produced similar release profiles in hydrochloric acid solution (pH 1.5) and lower release rates in water and PBS. For example, G4 and G5 PAMAM puerarin–dendrimer complexes released approximately 45% less puerarin compared to free puerarin after 3 hours in water (Figure 5). In addition, higher generation dendrimers tended to slow the release of puerarin. For example, G4 and G5 PAMAM puerarin–dendrimer complexes released approximately 25% less

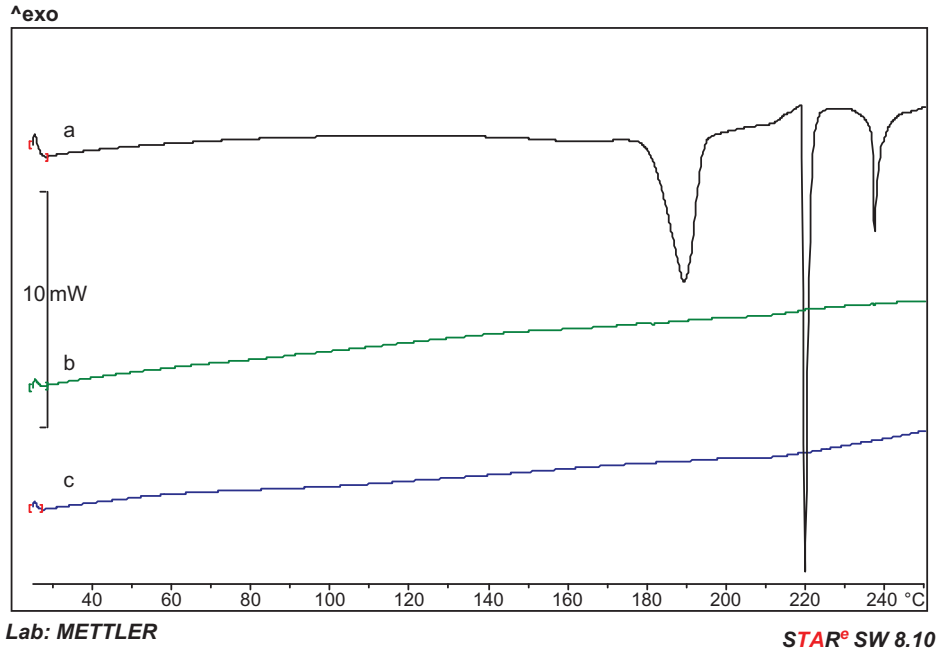

Figure 4. DSC thermograms of puerarin and puerarin–dendrimer complexes: (a) puerarin, (b) G3.5 PAMAM puerarin–dendrimer complex, (c) G4 PAMAM puerarin–dendrimer complex. Puerarin exhibited a sharp endothermic melting peak (T_m) at 190.3°C (a), but this peak was not present for G3.5 (b) and G4 PAMAM puerarin–dendrimer complexes (c).

puerarin than G3.5 and G4.5 PAMAM puerarin–dendrimer complexes after 3 hours in water (Figure 5).

Corneal permeation studies

Figure 6 and Table 1 display the corneal permeation profiles and parameters of puerarin–dendrimer complexes. PAMAM dendrimers did not increase the penetration rate of puerarin across the cornea (Table 1). It is worth noting that the cationic G4 PAMAM puerarin–dendrimer complex shortened the lag time of puerarin permeation across the cornea compared to the G3.5 PAMAM puerarin–dendrimer complex (0.32 versus 0.63 hours). Corneal hydration is frequently used as a parameter to evaluate damage to this tissue. Table 1 also indicated that the corneal hydration of puerarin–dendrimer complexes was below 80%, which suggests that PAMAM dendrimers did not damage the epithelium or endothelium during the experiments.

Determination of ocular residence time

To evaluate the interaction between the puerarin–dendrimer complexes and the cornea, ocular residence time of puerarin in puerarin–dendrimer complexes was compared to that of puerarin eye drops. Figure 7 displays the precorneal drug concentrations of puerarin–dendrimer complexes or free puerarin versus the concentration after instillation. The puerarin eye drops demonstrated rapid elimination with a half-life of 0.8 hour. The G3.5 and G4 PAMAM puerarin–dendrimer complexes showed prolonged residence time with elimination half-lives of 1.01 ± 0.14 hours and 1.80 ± 0.41 hours. The half-lives of the puerarin–dendrimer complexes were increased significantly higher than the puerarin eye drop.

Discussion

Drug can be either encapsulated or complexed to the dendrimer. Complexation ability of different amounts of drug in various dendrimers (PAMAM–G_n–NH_2 and PAMAM–$G_{n.5}$–$COOCH_3$) was studied to estimate the maximum number of puerarin molecules that can be incorporated in a dendrimer molecule. In this study, the drug-to-dendrimer ratios increased gradually from the G3.5 to the G5 PAMAM dendrimers, which suggests that –NH_2 group-terminated dendrimers were able to complex with puerarin more strongly than the –$COOCH_3$ group-terminated dendrimers. Some puerarin could be encapsulated into the core of the dendrimer with weak interactions influenced by the dendrimer generation and pH conditions. Full-generation PAMAM dendrimers possess primary amines on the surface and tertiary amines in their internal cavities, whereas half-generation dendrimers expose ester groups on their surface with internal tertiary amines. The reported pK_a values of the

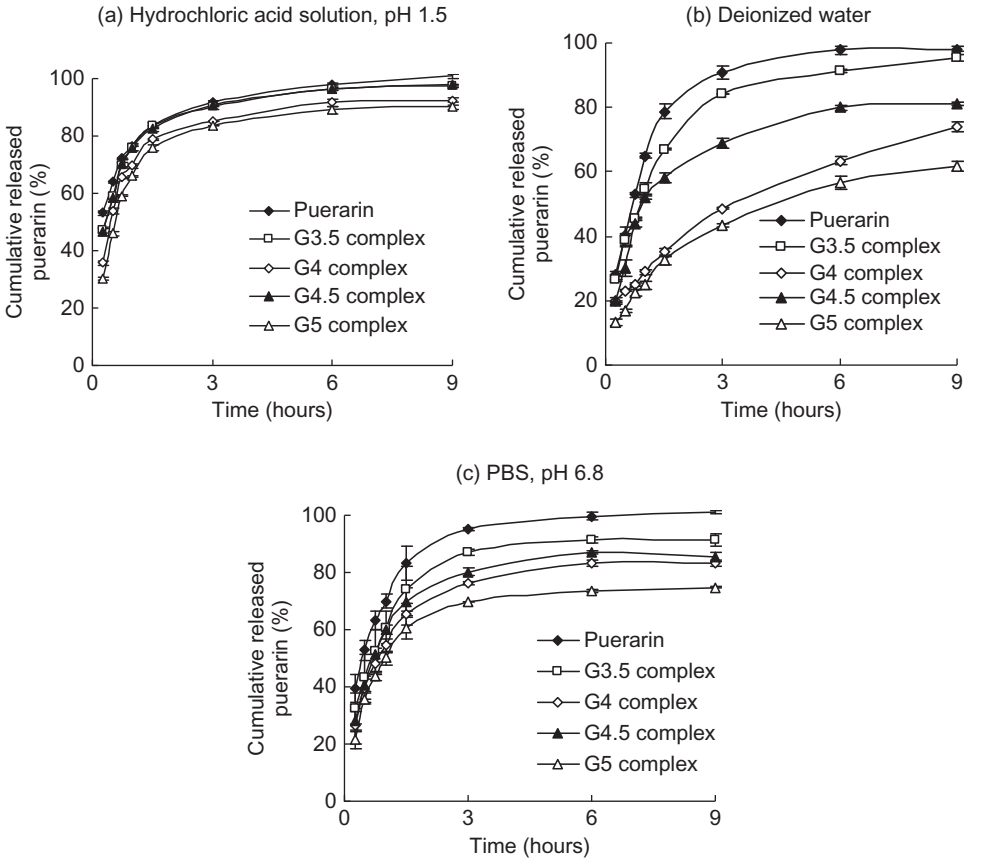

Figure 5. In vitro release of puerarin from puerarin–dendrimer complexes in various solutions. The puerarin–dendrimer complexes produced different release profiles in these three solutions. The complexes exhibit the most rapid release rate in (a) hydrochloric acid solution (pH 1.5), whereas the slowest release was in (b) deionized water, and (c) moderate release in PBS (pH 6.8). Data are presented as mean ± SD for three independent experiments.

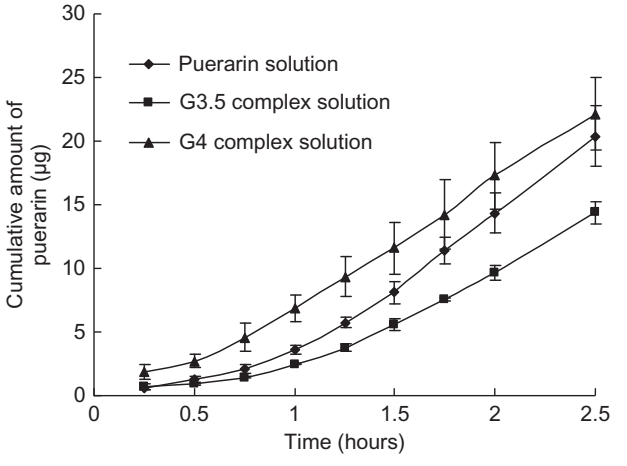

Figure 6. Corneal permeation of puerarin–dendrimer complex in rabbit corneas. Cumulative amount of puerarin from the puerarin solution and puerarin–dendrimer complex solutions that permeated the isolated rabbit corneas. Data are presented as mean ± SD for six independent experiments.

primary amines (surface groups) and the interior tertiary amines are 7.0–9.0 and 3.0–6.0 individually[28]. Most of the primary amines are protonated at pH 7.0 and all of the tertiary amines are protonated at pH 4.0. Therefore, the protonation level of the PAMAM dendrimers could be altered by pH changes, which in turn significantly altered the interaction of the PAMAM dendrimer with puerarin.

Puerarin, used as a therapeutic agent for cataracta glauca, is a weak acid with experimentally determined pK_a values of 7.4 and 10.0. The –OH-ionized functional group may act as a counterion for the dendrimer amine groups, thereby participating in the interaction between puerarin and the dendrimers. However, there was no significant increase in puerarin incorporated into the G3.5 PAMAM dendrimer. One possible reason may be the simple physical combination and/or the interaction between the internal tertiary amines of the dendrimers and the hydroxyl of the puerarin. Conversely, at pH 7.0–10.0, the puerarin hydroxyl group would be in its ionized form, and therefore the incorporation of puerarin into the G4 PAMAM dendrimer was enhanced, possibly because of the interaction between the positively charged dendrimer primary amines and the negatively charged puerarin hydrogen anion[28,29].

Table 1. Corneal hydration levels and cornea permeation parameters of puerarin in puerarin–dendrimer complexes.

	P_{app} ($\times 10^{-2}$, cm/h)	$Q_{2.5}$ (μg)	T_{lag} (hours)	HL (%)
Fresh cornea	—	—	—	78.31 ± 1.01
Puerarin	1.22 ± 0.27	20.16 ± 3.99	0.61 ± 0.06	78.97 ± 2.37
G3.5 complex	0.95 ± 0.14	14.66 ± 4.52	0.63 ± 0.06	79.10 ± 2.76
G4 complex	1.31 ± 0.08	22.12 ± 2.86	0.32 ± 0.05*	78.87 ± 2.04

$Q_{2.5}$, cumulative amount permeated in 2.5 hours; P_{app}, permeability coefficient; T_{lag}, lag time calculated by extrapolating the linear portion of the curve to the time axis; HL (%), $[1-(W_d/W_w)] \times 100$. Data are presented as mean ± SD for six independent experiments.
*$P < 0.05$ versus puerarin.

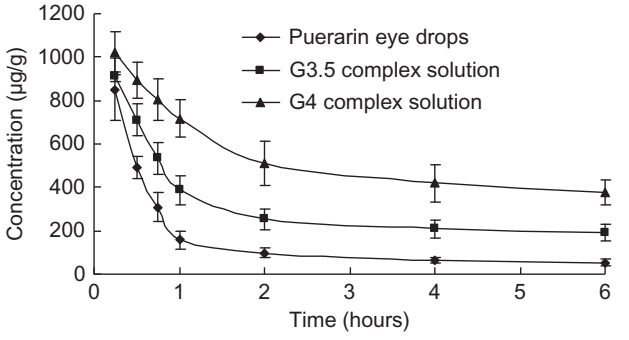

Figure 7. The concentration–time curve of puerarin in cornea. Ocular residence time of puerarin in puerarin–dendrimer complexes was compared to that of puerarin eye drops. The puerarin eye drops showed a rapid elimination with a half-life of 0.8 hour. The G3.5 and G4 PAMAM puerarin–dendrimer complexes showed prolonged residence time with elimination half-lives of 1.01 ± 0.14 hours ($P < 0.05$ versus puerarin) and 1.80 ± 0.41 hours ($P < 0.01$ versus puerarin). Data are presented as mean ± SD for seven independent experiments.

Furthermore, FTIR analysis supported the complex formation between PAMAM dendrimers and puerarin. The typical absorption bands of puerarin were covered and/or overlapped by pure G3.5 PAMAM dendrimer in the G3.5 PAMAM puerarin–dendrimer complex spectrum. Shifts to lower frequencies were obtained from the C=O stretching of the ester group (from 1398 to 1385 cm^{-1}) (symmetric) and the amide I band (C=O stretching from 1645 to 1631 cm^{-1}) of the G3.5 PAMAM dendrimer (because of hydrogen bonding between drug and dendrimers). The results could be explained by intermolecular hydrogen bonding between G3.5 PAMAM dendrimer groups and puerarin. In terms of G4 PAMAM, the O–H absorption bands of puerarin shifted to lower frequencies from 3396 to 3350 cm^{-1} and the N–H absorption bands of G4 PAMAM dendrimer shifted from 3366 to 3350 cm^{-1}. This could be explained by hydrogen bonding between G4 PAMAM dendrimer and puerarin. The amide I band (C=O stretching) of G4 PAMAM dendrimer shifted from 1641 to 1628 cm^{-1}, which indicates intermolecular hydrogen bonding between the G4 PAMAM dendrimer amide group and the puerarin molecule[30,31]. Overall, it can be concluded that puerarin complexed with PAMAM dendrimer primarily by hydrogen bonding.

The puerarin–dendrimer complexes exhibited different in vitro behavior in the different solutions. This is not surprising because puerarin would be virtually ionized in hydrochloric acid solution, thus weak interactions between puerarin and dendrimers would disappear. Higher ion concentrations in PBS also influenced the weak interaction between puerarin and PAMAM dendrimers, which resulted in the faster release rate of puerarin from puerarin–dendrimer complexes[22]. The release rate of puerarin was lower from full-generation dendrimers compared to that of half-generation puerarin–dendrimer complexes. It is known that the number of surface groups of PAMAM dendrimers increases exponentially with the increase in generation of dendrimers. This suggests that the strong interaction between puerarin and PAMAM dendrimers was responsible for the slow release rate of puerarin from the puerarin–dendrimer complexes. Overall, puerarin was released more slowly from puerarin–dendrimer complexes than free puerarin in deionized water and PBS (pH 6.8). The in vitro release rate of puerarin complexed with full-generation dendrimers was lower than that of half-generation dendrimers. From the above results, it can be concluded that PAMAM dendrimers could complex with puerarin by weak hydrogen-bonding interaction, and the slow release rate of puerarin from puerarin–dendrimer complexes resulted in the longer ocular residence time.

The corneal permeation studies suggested that there was no significant difference between puerarin–dendrimer complexes and puerarin eye drops on drug permeability coefficient. However, cationic G4 PAMAM dendrimer reduced the lag time of puerarin permeating the cornea. In addition, in PAMAM dendrimers and puerarin physical mixture, the cationic G4 PAMAM dendrimer enhanced the permeability coefficient of puerarin and increased the cumulative amount of puerarin permeating across the excised cornea (unpublished results). On the contrary, PAMAM dendrimer

can complex with puerarin to increase the solubility of puerarin. Furthermore, puerarin–dendrimer complexes showed longer ocular residence time in rabbits compared to puerarin eye drops. Because of puerarin eye drops rapidly eliminated by the lachrymal fluid, they were frequently administrated to maintain the appropriate drug concentration in aqueous humor to increase the drug ocular bioavailability, but which may cause both ocular and systemic side effects and poor compliance. The longer corneal residence time of puerarin–dendrimer complexes can finally improve the drug bioavailability and efficacy[32,33] and suggests a less frequent dosing rate in the future. These results suggest that puerarin–dendrimer complexes possess an obvious advantage in the drug therapy.

At physiological pH, positively charged G4 PAMAM dendrimers are expected to interact with the negatively charged corneas. Puerarin–dendrimer complexes did not demonstrate increased permeability compared to puerarin. This may be attributed to puerarin's slow release from puerarin–dendrimer complexes and delivery to the precorneal area. However, puerarin–dendrimer complexes prolonged the ocular residence time in rabbits, which can improve the drug bioavailability. To sum up, PAMAM dendrimer can act as drug carrier not only to increase the solubility of the drug but also to interact with the cornea to increase the drug corneal residence time, and finally to improve the drug ocular bioavailability. The optima formulation of puerarin–dendrimer complexes was selected based on the interaction between PAMAM dendrimer and the cornea and the drug concentration in vehicles as well as moderate release rate from puerarin–dendrimer complexes such as G4 PAMAM puerarin–dendrimer complex solution with 0.2% (w/v) G4 PAMAM dendrimer and 1% (w/v) puerarin.

The normal cornea has an HL of 78.31%. It has been reported that an HL of 3–7% units above normal (76–80%) denotes damage to the epithelium or endothelium[34]. In this study of puerarin–dendrimer complexes, the corneal HLs were lower than 80%, suggesting no damage to the epithelium or endothelium. Fischer et al. demonstrated that the magnitude of cytotoxic effects of several cationic polymers was found to be concentration- and time-dependent[35]. Vandamme and Brobeck have assessed ocular tolerance following the application of dendrimer test product to the eyes of three adult male New Zealand albino rabbits. The results showed that dendrimers were classified as weakly irritant products for the eye at a concentration of 2.0% (w/v) or lower, and the aqueous polymer solutions of dendrimers did not cause any ocular irritation[20]. Further studies are in progress on attaching some chains to shield the overall positive charges on cationic dendrimers to further reduce the toxicity of PAMAM dendrimer[36].

Conclusions

Puerarin efficiently complexes with PAMAM dendrimers, primarily through hydrogen-bonding interactions. Puerarin was released more slowly from puerarin–dendrimer complexes than the free puerarin in PBS (pH 6.8). The puerarin–dendrimer complexes exhibited longer ocular residence time in rabbits than puerarin eye drops, without damage to the corneal epithelium or endothelium. PAMAM dendrimers are thus a promising ocular drug carrier that may improve drug bioavailability and efficacy.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 30873447).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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