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Stealth dendrimers for antiarrhythmic quinidine delivery

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Abstract Dendrimers have been attracting growing attention because of their unique well-defined globular nanoscale architecture and numerous functional groups on the surface. Attachment of PEG to the dendrimer generates stealth dendrimers, which have promising structural advantages for drug delivery. In this study, synthetic methods were explored to deliver antiarrhythmic quinidine by stealth dendrimers. In particular, quinidine was covalently attached to anionic G2.5 and cationic G3.0 polyamidoamine (PAMAM) dendrimers via a glycine spacer, respectively. The resulting quinidine-PAMAM-PEG conjugates were characterized and confirmed by FT-IR and ^{1}H-NMR. In vitro hydrolysis was carried out in pH 7.4 PBS buffer at 37 °C to confirm the bioavailability of the conjugated quinidine.

Introduction

Polymeric drug delivery carriers play an essential role in drug delivery. However, many of polymers exhibit poor drug loading efficiency because of their limited functional groups on the backbone for drug loading. As a consequence, an increased amount of polymer carrier along with the drug is required for in vivo applications. Although those polymers are believed to be safe, long-term use of drugs delivered with those polymers may lead to accumulation with adverse effects. To prevent or slow down the unfavorable accumulation of residual drug carriers in the body, it is highly demanded to develop polymer drug delivery systems with improved drug loading capacity. Dendrimers are highly branched, nanoscale macromolecules with low polydispersity and high functionality [1, 2]. Compared to linear polymers, dendrimers have more reactive surface ending groups, making dendrimers an ideal drug carrier to create high-loading capacity controlled release devices [3]. In addition, dendrimers can be tailored to deliver different drugs and engineered to obtain control over the loading degree and release rate of the delivered drugs. Dendrimers as drug delivery carrier have been investigated to deliver genes [4], antisense oligonucleotides [5], peptides [6, 7], proteins [8], small-molecular-weight drugs [9, 10], imaging contrast agents [11, 12]. In our prior work, we have demonstrated that drugs can be either covalently conjugated to polyamidoamine (PAMAM) dendrimer via a PEG spacer [10] or physically encapsulated inside the PEGylated dendrimer core [13].

Attachment of PEG to the dendrimer generates stealth dendrimers, which have shown many promising structural advantages for drug delivery. For instance, well-known stealth properties of PEG can reduce cytotoxicity and immunogenicity of dendrimers and provide stealth dendrimers with excellent solubility in both aqueous and most organic solutions as well as favorable pharmokinetic and tissue distribution. Additionally, presence of PEG can improve the enzymatic stability of coupled drugs by steric hindrance [7]. In this study, we focused on extending

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application of stealth dendrimers to quinidine delivery. Quinidine is an antiarrhythmic drug for the correction of abnormal heart rhythms and administered orally. Quinidine contains a hydroxyl functional group, which, however, is sterically hindered by adjacent quinoline and peperidine groups. The embedded hydroxyl group makes quinidine hardly react with a traditional polymer molecule because of its limited functional groups, thus leading to poor drug loading efficiency. To overcome steric hindrance and improve loading efficiency, a short glycine spacer was added to extend the quinidine's hidden hydroxyl group. In particular, quinidine-loaded stealth dendrimer drug carriers were constructed based on anionic G2.5 and cationic G3.0 PAMAM dendrimers, respectively. Accordingly, two synthetic approaches were proposed and discussed. The resulting conjugates were characterized with ^{1}H-NMR and FT-IR. In vitro release study was conducted to confirm the bioavailability of the modified quinidine.

Materials and methods

Materials

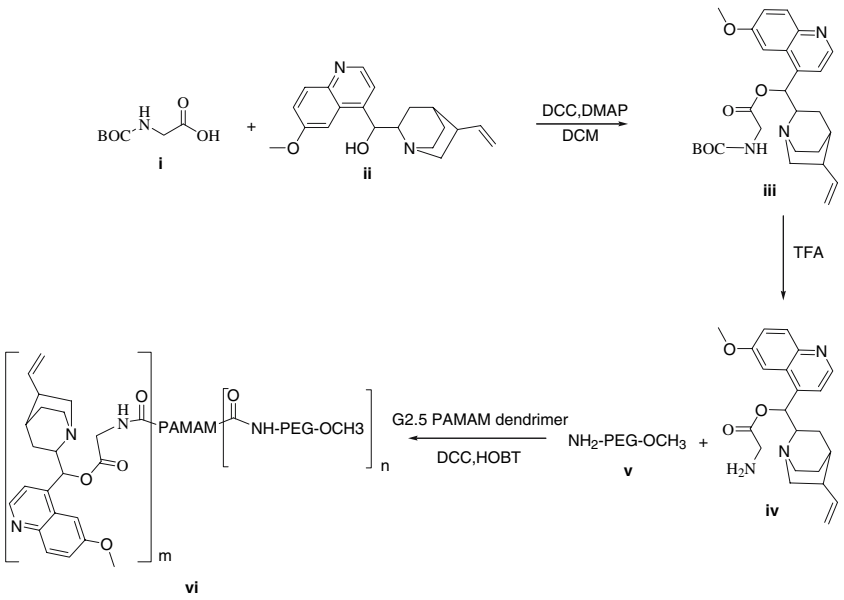
Starburst^{TM} G2.5 and G3.0 PAMAM dendrimers, methoxypoly(ethylene glycol) (MPEG-OH, Mn = 2,000), *O*-(2-aminoethyl)-*O′*-methylpolyethylene glycol (MPEG-NH_2, Mn = 2,000), quinidine, N-t-BOC-glycine, 1-hydroxybenzotriazole (HOBT), dicyclohexyl-carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), *N*,*N′*-disuccinimidyl carbonate (DSC), triethylamine (TEA), hydrochloric acid (HCl), trifluoroacetic acid (TFA), tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF), and diethyl ether were purchased from Sigma-Aldrich and used as received. Fresh distilled water was used throughout the experiment. Deuterium oxide (D_2O, 99.9%) was obtained from Cambridge Isotope Laboratories.

Spectroscopic characterization

FT-IR spectra were obtained on a Nicolet Nexus 670 spectrometer. UV/Visible absorbance data were gained on a Beckman DU640 Spectrophotometer. ^{1}H-NMR spectra were recorded on a 300 MHz Varian Gemini 300 MHz spectrometer. Prior to measurement, 5 mg of each sample was dissolved in the deuterated solvent, filtered and degassed. The chemical shift of D_2O is 4.8 ppm.

Synthesis of quinidine-PAMAM (G2.5)-PEG conjugates

The synthesis route is presented in Scheme 1. To 3 mL of DCM were added 0.8 g of quinidine (2.5 mmol), 0.44 g of BOC-glycine (2.5 mmol), and 0.31 g of DMAP (2.5 mmol). Then the solution was mixed with 4 mL of DCM containing 0.52 g of DCC (2.5 mmol). The mixture was stirred overnight. Dicyclohexyl urea (DCU) produced in the reaction was filtered off. The solvent was evaporated and the residue was extracted by acetone, filtered, and dried. BOC-glycine-quinidine conjugates were dissolved in 5 mL of DCM, and then 5 mL of 90% TFA was added to the solution. The solution stood at room temperature for 30 min to remove BOC groups. The residue obtained by evaporation was taken up in DCM and then washed with saturated sodium bicarbonate. The purified BOC-glycine-quinidne was obtained by removing the solvent.



Scheme 1 Synthesis of quinidine-PAMAM (G2.5)-PEG conjugates

Starburst™ dendrimer was dried to remove methanol using rotary evaporation. Then 100 mg of G2.5 Starburst™ PAMAM dendrimer (34 μmol) was dissolved in water and then converted to the free acid form by acidification with 5 N hydrochloric acid to pH 1.0. The acidified solution was evaporated to dryness under vacuum. The dendrimer in free acid form was dissolved in 4 mL of DMF. The dendrimer was added to 3 mL of DCM containing 250 mg of glycine-conjugated quinidine, 136 mg of MPEG-NH_2, 14 mg of DCC, and 9.2 mg of HOBT. The mixture was continuously stirred for 2 h. The reaction system was stirred 14 h. DCU precipitated during the reaction was removed by centrifugation. The mixture was dried under vacuum. It was dissolved in acetone followed by precipitation in ether/hexane. Further separation of PEG-PAMAM conjugates from unreacted PEG and small molecule reagents was conducted by ultrafiltration method using an Amicon® stirred cell with an YM10 membrane against fresh distilled water. The product was characterized by FT-IR and ^{1}H-NMR.

Synthesis of quinidine-PAMAM (G3.0)-PEG conjugates

The synthesis of quinidine-PAMAM-PEG conjugates is described in Scheme 2. Glycine-conjugated quinidine was first synthesized following the procedure described in the previous section. About 35 mg of DSC was dissolved in 4.5 mL of DMF. A DMF solution (1.8 mL) containing dissolved 30 mg of G3.0 PAMAM was added dropwise to the DSC solution over 1 h. TEA (17.6 μL) was slowly added to the solution while stirring. After 12-h reaction, the solution was precipitated with cold ether and vacuum dried to obtain G3.0 PAMAM-NHS esters. The obtained PAMAM-NHS esters were further coupled with glycine-conjugated quinidine and MPEG-NH_2 in 2 mL of 0.1 M biocarbonate solution. The reaction was allowed to proceed overnight. The product was purified using ultrafiltration. The filtrate was lyophilized, and then characterized with FT-IR and ^{1}H-NMR.

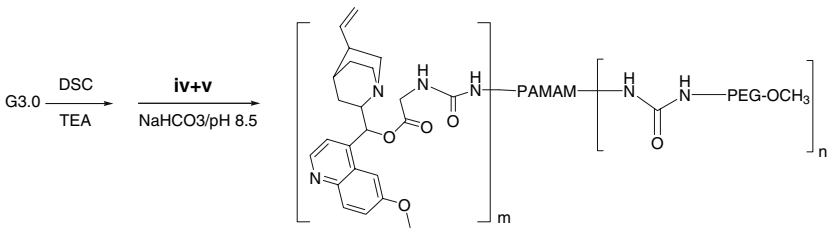
In vitro hydrolysis study

To prove the bioavailability of the conjugated quinidine, in vitro hydrolysis of quinidine-PAMAM-PEG was conducted. The quinidine-PAMAM-PEG conjugates were incubated in a dialysis bag with MWCO 3500 in PBS buffer (pH 7.4) at 37 °C. Then, 3 mL of drug release medium was taken out at each pre-determined interval and measured at 254 nm by a UV–VIS spectrophotometer. Drug release medium was put back immediately after each measurement. The mass ratio of drug released, M_t/M_0 (M_t, the accumulated mass of drug released at time t; M_0, the original mass of drug loaded), was quantified as a function of time. The hydrolyzed product was collected from inside the dialysis bag, dried, and then characterized with ^{1}H-NMR.

Results

Half and full generation Starburst™ PAMAM dendrimers have carboxylate and primary amine groups on the surface, respectively. Two synthetic approaches were explored to couple quinidine to G2.5 and G3.0 PAMAM dendrimers via a glycine spacer. Glycine is a nonpolar amino acid. Prior to the drug-dendrimer coupling reaction, BOC-glycine was conjugated to quinidine through an ester bond. The resultant BOC-glycine-quinidine conjugates were treated with TFA to remove BOC. Primary amine group containing PEG and glycine-extended quinidine reacted with the carboxylate groups of G2.5 PAMAM dendrimer to yield amides using the DCC/HOBT method (Scheme 1). In the case of G3.0 PAMAM dendrimer as drug carrier (Scheme 2), DSC reacted with the primary amine groups on the dendrimer surface to generate amine-reactive NHS esters. The DSC-activated dendrimers (i.e., NHS esters) were then reacted with amine containing PEG and glycine-extended quinidine to yield ureas (i.e., quinidine-PAMAM-PEG conjugates). The structure of the conjugates was confirmed by ^{1}H-NMR (Fig. 1).

Scheme 2 Synthesis of quinidine-PAMAM (G3.0)-PEG conjugates



The quinidine-PAMAM-PEG conjugates were characterized with FT-IR spectroscopy. The FT-IR spectrum (Fig. 2a) of unmodified G2.5 shows a broad adsorption band (peak A) between 4,000 and 2,500 cm^{-1} due to amide N–H stretching absorption and C–H stretching vibrations. In addition, unmodified G2.5 shows a strong carbonyl adsorption band (peak B) at 1,662 cm^{-1}, which corresponds to the carboxylate groups on the periphery of the

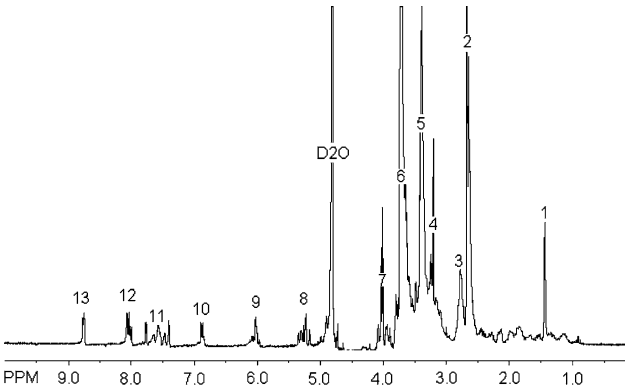


Fig. 1 ^{1}H-NMR spectrum of quinidine-PAMAM (G2.5)-PEG conjugates. (Peak 6 from repeat unit of PEG; peak 10–13 from quinoline of quinidine, peak 8 and 9 from ethylene of quinidine, peak 7 from methyl of quinidine, peak 1 from piperidine of quinidine; peak 2–5, from repeat unit of PAMAM)

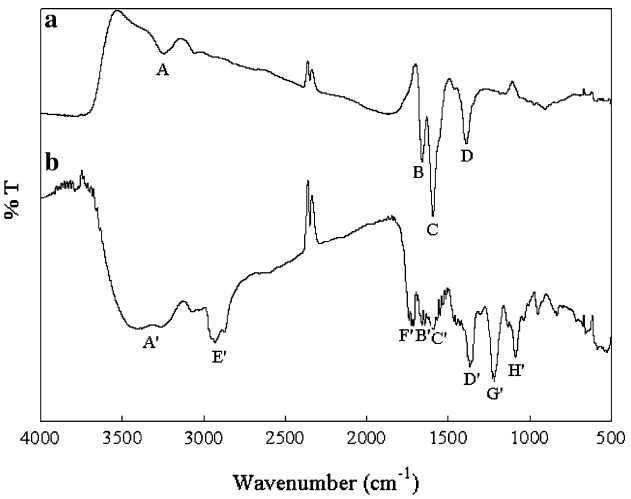


Fig. 2 FT-IR of (**a**) G2.5 PAMAM dendrimer and (**b**) quindine-PAMAM (G2.5)-PEG conjugates

dendrimer. The peak C at 1,597 cm^{-1} and the peak D at 1,625 cm^{-1} are assigned to N–H bending vibrations and C–H bending vibrations, respectively. The other minor peaks in the region below 1,100 cm^{-1} are the vibrations of the benzene rings of quinidine and out-of-plane N–H wagging of quinidine and dendrimer. Upon the completion of the coupling reaction, the FT-IR spectrum (Fig. 2b) of quinidine-PAMAM-PEG shows a strong C–O stretching band at 1,092 cm^{-1} and an O–H stretching adsorption band at 2,939–2,896 cm^{-1} indicating the appearance of PEG. An adsorption band at 2,939–2,896 cm^{-1} is assigned to C–H stretching vibrations of the conjugated PEG and quinidine. A similar analysis was applied to the FT-IR spectrum of G3.0 PAMAM-based quinidine-PAMAM-PEG, and the spectroscopic analysis confirmed that the conjugation reaction was successful.

The bioavailability of the conjugated quinidine was examined though in vitro release study. Since an ester bond links quinidine and glycine in the conjugates of quinidine-PAMAM (G2.5)-PEG and quinidine-PAMAM (G3.0)-PEG, this bond can be easily cleaved to release quinidine. The release kinetics of quinidine from drug-dendrimer complex was photometrically determined. At 8 h, 50% of the conjugated drug was released. At 24 h, the hydrolyzed product was collected, dialyzed, and analyzed using ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectrum clearly showed the disappearance of all proton peaks of quinidine, indicating quinidine was completely released from the conjugates within 24-h in vitro hydrolysis.

Discussion

A high drug loading achieved by coupling drug molecules to the dendrimer surface may improve therapeutic effectiveness significantly at the molecular level. Hydroxyl group is the only functional group within quinidine available to the coupling reaction. However, it is sterically hindered by the adjacent quinoline and peperidine groups. Direct coupling of quinidine to the dendrimer surface resulted in a low drug loading as the steric hindrance allows the embedded hydroxyl group little reactivity and impairs quinidine's binding to the dendrimer surface. In this study, quinidine was first directly coupled with PAMAM using the DCC/DMAP method. Quinidine loading was estimated using ^{1}H-NMR spectroscopy. An average of two quinidine molecules were covalently coupled with a G2.5 PAMAM dendrimer molecule using the direct coupling strategy. According to the de Gennes dense packing effect, the end groups become more densely packed as a dendritic molecule grows larger, and eventually the dendrimer reaches its upper generation limit [14]. Consequently, conjugated quinidine molecules on the dendrimer surface may cause even more serious steric hindrance that prevents further coupling PEGs to the dendrimer surface sites. Glycine, an amino acid, was employed as an extension to the hydroxyl group of quinidine. Five quinidine molecules were loaded onto the dendrimers surface via a glycine spacer, although not significantly. The glycine-extended hydroxyl group had less steric hindrance for reaction than the unmodified hydroxyl group, thereby allowing quinidine to react with the dendrimer surface group more easily. The glycine spacer would give more flexibility to the surface-bound quinidine molecules, allowing PEG to access the unmodified dendrimer surface sites near the conjugated quinidine molecules. The above results indicate that the steric hindrance of drugs could be overcome through introduction of an appropriate spacer. After a spacer is introduced, the resulting spacer-extended drugs have less steric hindrance

and can react with dendrimer more easily to create high drug loading carriers. Furthermore, spacer-bound drugs produce more flexible space, making PEGs access the unmodified surface groups near the occupied sites for PEGylation more easily. Glycine is the simplest of the 20 standard amino acids. Similarly, glycine spacers of high aggregation degree, e.g., diglycine, triglycine, hexaglycine, can also be applied to enhance drug loading on the dendrimer surface following the synthesis methods presented herein. However, for optimal drug delivery by stealth dendritic drug carriers, such factors as drug and PEG loading degrees per dendrimer, the aggregation degree of glycine, length of PEG, and generation of dendrimer must be considered. The in vivo and in vitro effects of above factors need to be investigated systematically. Our future work will focus on engineering and biological evaluation of such stealth dendritic drug delivery systems for optimal drug delivery.

Conclusions

Quinidine was successfully coupled to half (G2.5) and full (G3.0) generations of stealth dendrimers via a glycine spacer. This study has demonstrated that the embedded functional groups like quinidine's hydroxyl group could be extended by a glycine spacer and delivery of such drugs could be realized by stealth dendrimers with improved loading efficiency.

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