Nanoparticulate Drug Delivery Systems Based on Hydrotropic Polymers, Dendrimers, and Polymer Complexes

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ABSTRACT

Nanoparticulates, composed of hydrotropic polymers, dentrimers, or polymer complexes, were developed to deliver poorly water-soluble drugs. Nanoparticulate polymer systems were also developed for encapsulation of proteins. Hydrotropic amphiphilic block copolymers self-associated to form nanosized micelles in an aqueous solution. The hydrotropic micelles containing hydrotrope-rich cores exhibited not only high loading capacity of paclitaxel but also enhanced physical stability in aqueous media. Polyglycerol dendrimers increased the water solubility of paclitaxel by several orders of magnitude due to the presence of a high local density of ethylene glycol units having the hydrotropic property. The hydrogel nanoparticles were also prepared by simple mixing of two polymer solutions.

Keywords: hydrotropic copolymer, dendrimer, hydrogel, polymer micelle

1 INTRODUCTION

During the last few decades, a number of methods have been developed to prepare polymeric nanoparticles, primarily based on chemical crosslinking of monomers [1], self-assemblies of amphiphilic polymers [2], and ionic complexation between oppositely charged polyelectrolytes [3]. Recent studies have shown that such nanoparticles are important as drug delivery systems due to their ability to imbibe a variety of therapeutic drugs, protect the drugs from harsh environments in the body, and deliver the drugs into the specific sites of the body [4].

Formulation of poorly water-soluble drugs and proteins has been one of the most important issues in the field of drug delivery. Although therapeutic protein and peptides have highly specific and potent biological functions, their delivery has been limited to parenteral administration and frequent injection is required due to their short half lives in the blood. The simple method of preparing micro- or nanoparticles to encapsulate protein drugs for long-term delivery is obviously needed for patient compliance and convenience.

Paclitaxel, a hydrophobic drug, is an anticancer agent effective against a wide range of tumors, whereas its clinical applications have been limited by its extremely low solubility in the biological solution (< 1 μg/ml). Although paclitaxel is commercially available in formulation based on 50:50 mixture of Cremophor EL (caster oil modified with PEG) and ethanol, this formulation has been reported to cause hypersensitivity reactions, sometimes fatal, primarily ascribed to cytotoxicity of the amphiphilic compound and the organic solvent [5]. The current strategies for formulation of paclitaxel include surfactant micelles, polymeric micro- or nanoparticles, solid dispersion, and cosolvents.

Hydrotropic agents are water-soluble compounds capable of enhancing water solubilities of poorly soluble drugs. They self-associate and form noncovalent assemblies of nonpolar microdomains to solubilize hydrophobic solutes at the minimal hydrotrope concentration. The self-aggregates of hydrotropes have the unique three-dimensional architecture, such as planar or open-layer structures, instead of forming compact core-shell type aggregates. The application of hydrotropic agents, however, has not been practical because they can dissolve poorly-soluble drugs at high concentrations, which may lead to a significant absorption of hydrotropic agents along with the drug. Recently, we have identified a number of hydrotropic agents that increase the water-solubility of paclitaxel by several orders of magnitude [6].

In this study, we have developed nanoparticulate drug delivery systems for poorly water-soluble drugs and proteins. As the potential delivery systems of paclitaxel, we prepared novel amphiphilic block copolymers and dendritic polymers. The block copolymers were synthesized using poly(ethylene glycol) (PEG) and hydrotropic segments such as poly(2-(4-vinylbenzyloxy)-*N*,*N*-diethylnicotinamide) (PDENA). Hydrotropic dendrimers, composed of polyglycerol, were synthesized by step-by-step allylation and dihydroxylation reactions. For protein encapsulation, hydrogel nanoparticulate systems were developed based on the physical interactions, such as hydrogen bonding and electrostatic interactions.

2 MATERIALS AND METHODS

2.1 Hydrotropic Block Copolymers

Monomethoxy PEG (Mn = 5000) and triethylamine were placed into a two-neck round flask and dissolved in methylene chloride. To this solution, bromopropionyl bromide was added dropwise with gentle stirring at 0 ^{o}C.

The reaction mixture was kept at room temperature for 24 h, and then precipitated in cold diethyl ether to obtain PEG-Br.

PDENA-PEG block copolymers were synthesized by atom transfer radical polymerization (Figure 1). In brief, DENA, Cu(I)Br, and PEG-Br were added to a round-bottom flask, which was then evacuated and refilled with dry nitrogen. To this reaction flask were added toluene and N, N, N', N',N"-pentamethyldiethylenetriamine. The reaction was performed at 85 ^{o}C with vigorous stirring for 3 h. The resulting solution was diluted with methylene chloride, passed through a silica gel column to remove the copper catalyst, and precipitated in cold diethyl ether.

Paclitaxel-loaded polymer micelles were prepared by a dialysis method. The block copolymer was dissolved in acetonitrile and a predetermined amount of paclitaxel was added to the polymer solution. The mixture solution was stirred for 6 h and dialyzed against distilled water.

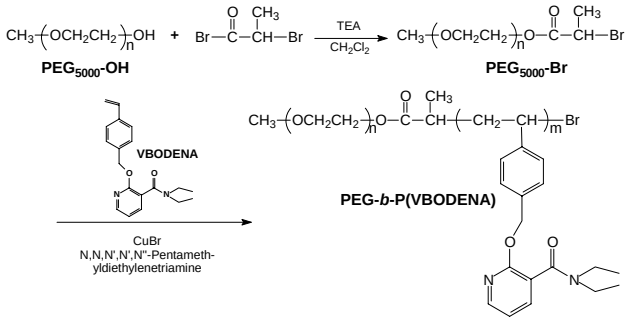

Figure 1. Synthesis of PDENA-PEG block copolymer.

2.2 Hydrotropic Dendrimers

Polyglycerol dendrimers (PGDs) with generations 3, 4, and 5 were synthesized by a two-step process based on allylation of alcohols and catalytic dihydroxylation (Figure 2) [7]. For example, to prepare PGD with generation 5 (PGD-5), allylation of PGD-4 was carried out as follows: the predetermined amount of PGD-4 was dissolved in 50% NaOH with heating, to which tetrabutylammonium bromide (TBAB) was added and dispersed under vigorous stirring using the mechanical stirrer. After mixing with toluene, the organic phase was separated from the mixture, dried over $MgSO_4$, filtered, and concentrated using a rotary evaporator. The solution was then purified by column chromatography (silica gel, petroleum ether/ethyl acetate 10:1 to 1:1) to obtain PGD-4.5. In the next step, PGD-4.5 and *N*-methylmorpholine *N*-oxide (NMO) were dissolved in the mixture of distilled water, acetone, and *t*-butanol. After adding the aqueous OsO_4 solution, the reaction was continued with stirring for 20 h at room temperature. Thereafter, all the volatile compounds were removed under vacuum, and the crude products were purified by dialysis in methanol. The resulting solution was concentrated in vacuo, and water was added to dissolve the products. Water-insoluble impurity was removed via passing the syringe filter (0.45 μm in pore size), followed by lyophilization.

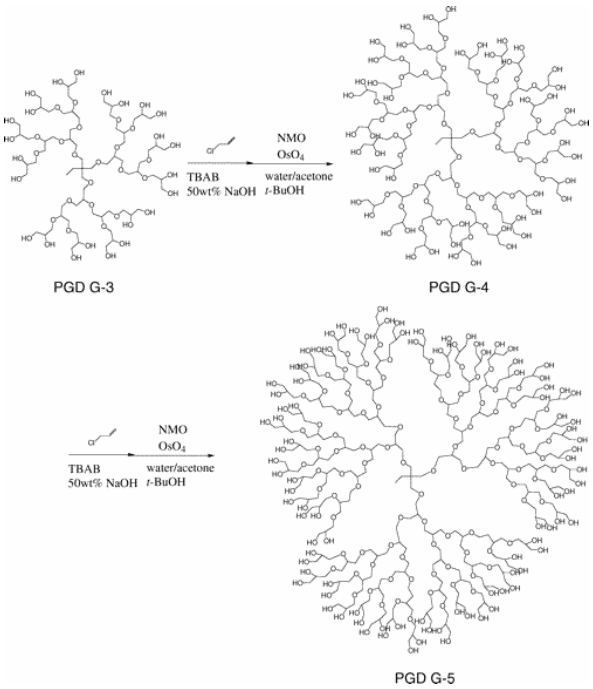

Figure 2. Synthesis of hydrotropic polyglycerol dendrimers with generations 3, 4 and 5. (From reference 7)

2.3 Hydrogel Nanoparticles

Hydrogel nanoparticles specifically designed for protein delivery have been developed based on physical interactions between two polymers in an aqueous solution. The nanosized particles were formed by simple mixing of two polymer solutions through which physical crosslinking occurs via hydrogen bonding or electrostatic interactions. For example, chitosan solution containing a protein was added dropwise into a solution of tripolyphosphate (TPP). Other polymer systems were also used to prepare hydrogel nanoparticles containing either lysozyme or albumin as a model protein.

The morphology of nanoparticles was observed by using a Philips CM 100 which was operated at an accelerating voltage of 80 kV. A drop of sample solution was placed onto a copper grid, and the nanoparticles were negatively stained by 2% uranyl acetate solution. The particle size was measured by dynamic light scattering. The intensity autocorrelation was obtained at a scattering angle of 90^{o} at 25 ^{o}C. The hydrodynamic diameter of nanoparticles was calculated by the Stokes-Einstein equation.

The encapsulation efficiency of lysozyme was estimated using a bicinchoninic acid (BCA) assay. The nanoparticle suspension was centrifuged at 10,000 rpm for 10 min. The clear supernatant was used for the determination of lysozyme amount that is not encapsulated.

3 RESULTS AND DISCUSSION

PDENA-PEG block copolymers were obtained by atom transfer radical polymerization of DENA monomers in the presence of macroinitiator, PEG-Br. The block length of the hydrotropic polymer was readily controlled by adjusting the initial feed ratio of DENA monomer to PEG-Br, and the molecular weights of the block copolymers were in a good agreement with the theoretical values. These hydrotropic amphiphilic block copolymers self-assembled to form micellar structures in an aqueous solution. The mean diameter of micelles was in the range of 30-50 nm which was dependent on the block length of the hydrotropic polymer. The critical micelle concentrations (CMCs) of the block copolymers were higher by an order of magnitude than those of other typical polymer micelles based on poly(D,L-lactide) (PLA)-PEG and poly(phenylalanine) (PPA)-PEG. This is presumably due to less hydrophobic nature of the hydrotropic block.

The paclitaxel loading by the dialysis method was performed by varying the feed ratio of drug to polymer to determine the maximum loading capacity. The result showed that the highest loading content of paclitaxel was 37.4 wt% for hydrotropic micelles. Loading of paclitaxel into the micelles increased the size to 100-120 nm. On the other hand, PLA-PEG copolymer could imbibe paclitaxel up to 27.6 wt% by the solid dispersion technique. It is interesting to note that drug-loaded PLA-PEG polymer micelles were precipitated within 3 days because they were unstable in an aqueous solution, whereas the hydrotropic micelles maintained the paclitaxel content for more than 30 days (Figure 3) [8]. This indicated that the poor physical stability of paclitaxel-loaded micelles can be overcome by introducing hydrotropic property into the core structure of the polymer micelle. Further, after being freeze-dried, hydrotropic micelles were easily redissolved in aqueous media by applying a simple vortexing process with or without a mild heating at 60 ^{o}C for 1 min.

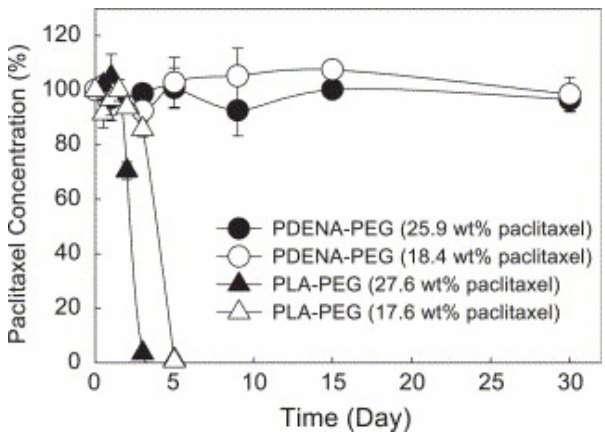

Figure 3. Changes in paclitaxel concentrations of polymer micelles in distilled water. (From reference 8)

In vitro release profiles of paclitaxel from polymer micelles were examined in an aqueous solution containing 0.8 *M* sodium salicylate (Figure 4). For PDENA-PEG copolymers, the micelle with 25.9 wt% loading amount released most drugs within 48 h, whereas the micelle with 31.3 wt% showed complete release within 24 h. Since the micelle with the higher drug content has lower polymer concentration, there is less polymer-drug interaction in the micelles, resulting in faster release kinetics. The result also showed that paclitaxel was released faster from the hydrotropic polymer micelles than from PLA-PEG micelles. The lower hydrophobicity of hydrotropic micelles, as demonstrated by the CMC values, might make the paclitaxel release from the micelles easier.

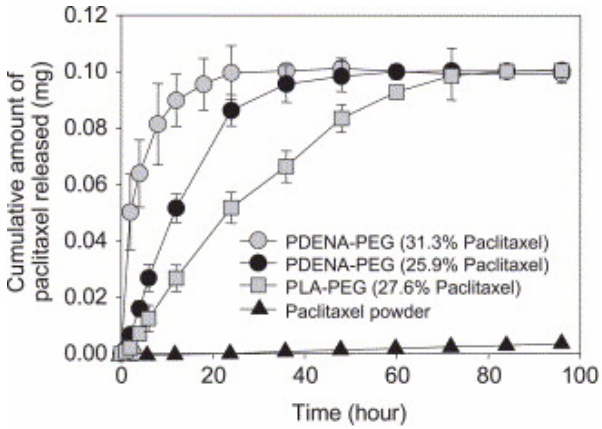

Figure 4. *In vitro* release profile of paclitaxel from polymer micelles in a 0.8 M sodium slicylate solution at 37 ^{o}C. The total amount of drug loaded was 0.1 mg. (From reference 8)

As an alternative method to hydrotropic polymeric micelles, we have prepared hydrotropic dendrimers, composed of ethylene glycol units. PEGs have been frequently used for increasing the water solubility of poorly soluble drugs. In particular, PEG with the molecular weight of 400 (PEG 400) has been investigated as a cosolvent for various hydrophobic drugs [9]. PEG 400 is known to self-associate through hydrogen bonding at high concentration regions (>80%), which may alter water structure to influence the water solubility of poorly soluble drugs. However, the use of such high concentrations of PEG 400 has limited biomedical applications. Since the high density of PEG 400 or ethylene glycol units is the key factor in increasing the solubility of poorly water-soluble drug, it was of interest to design new ethylene glycol-based polymer architectures which provide a high local density of the ethylene glycol unit. Thus, we prepared the hydrotropic polyglycerol dendrimers (PGDs) with different generations that were expected to provide higher local density of ethylene glycol units than linear PEGs.

The effect of PGDs on paclitaxel solubility in water was examined by determining the paclitaxel concentration using HPLC. As shown in Figure 5, paclitaxel solubility in all

PGDs was much higher than that of PEG 400. At 10 wt% of PEG 400 in distilled water, the paclitaxel solubility was 0.4 μg/ml. On the other hand, at the same concentrations of PGDs, paclitaxel solubility was in the range of 80-128 μg/ml which is 3 orders of magnitude higher than that in pure water (0.3 μg/ml). These results suggest that the increased solubility of paclitaxel in water is due to the dendritic architecture that provides hydrotropic effect by local high concentrations of ethylene glycol units.

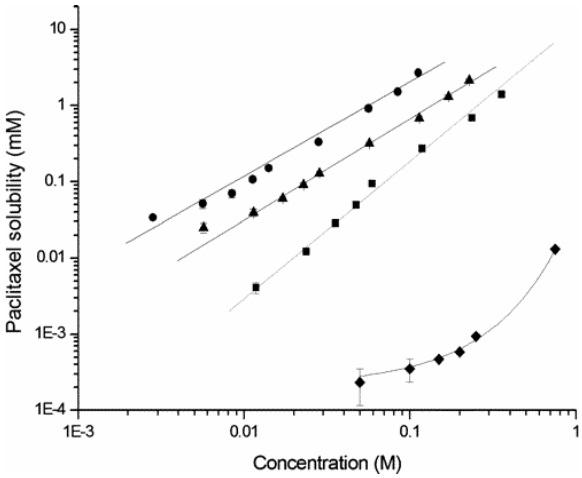

Figure 5. Water solubility of paclitaxel as a function of the PGD concentration: (●) PGD-5; (▲) PGD-4; (■) PGD-3; (◆) PEG 400. (From reference 7)

In vitro release profiles of paclitaxel from PGDs were examined in a PBS solution containing 0.15 *M* sodium salicylate (Figure 6) [10]. All of the paclitaxel was released from dendrimer solutions within 96 h, whereas the release of paclitaxel from its particulate state (i.e., in the absence of PGDs) was significantly slower.

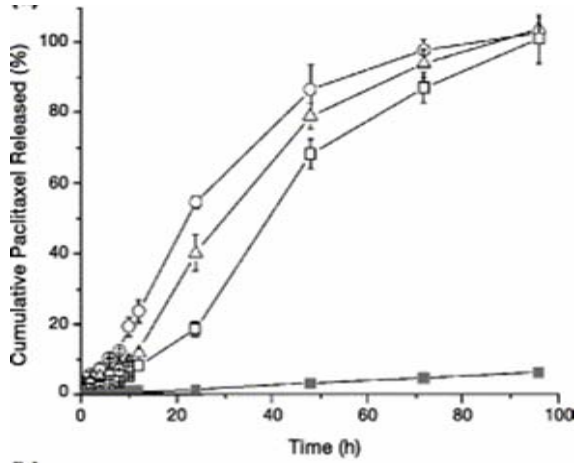

Figure 6. Cumulative release profiles of paclitaxel from dendrimer solutions: (○) PGD-5 (80%); (□) PGD-4 (80%); (△) PGD-3 (80%); (■) paclitaxel particle. (From reference 10)

The higher paclitaxel release rates at higher generations of PGDs might be due to the differences in their ability to solubilize paclitaxel.

Hydrogel nanoparticles for protein delivery were also prepared. As shown in Figure 7, nanosized particles (50-100 nm in diameter) containing a protein (lysozyme in this case) could be prepared by mixing two polymers under an optimized condition. In this particular system, more than 95% of lysozyme was encapsulated into the nanoparticles. This simple method to encapsulate protein is highly promising to tailor the delivery system for a specific protein.

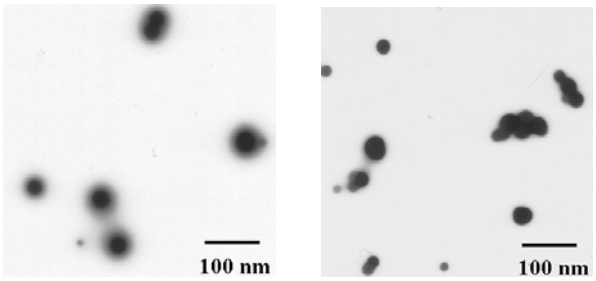

Figure 7. TEM images of hydrogel nanoparticles. The samples were stained with 2% uranyl acetate solution.

4 CONCLUSION

Polymeric nanoparticulate systems for drug delivery were developed using hydrotropic block copolymers, dendritic polymers, and hydrogels. These nanoparticles have shown great potentials for delivery of poorly water-soluble drugs as well as proteins.

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