PHARMACEUTICAL NANOTECHNOLOGY

Evaluation of Phenylbutazone and Poly(amidoamine) Dendrimers Interactions by a Combination of Solubility, 2D-NOESY NMR, and Isothermal Titration Calorimetry Studies

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ABSTRACT: The interactions between phenylbutazone, a well-established nonsteroidal anti-inflammatory drug, with different generations of poly(amidoamine) (PAMAM) dendrimers, have been investigated by aqueous solubility, two dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) and isothermal titration calorimetry (ITC) studies. Solubility results showed that PAMAM dendrimers significantly enhanced the aqueous solubility of phenylbutazone and the solubilization was much influenced by dendrimer concentration, generation, surface function group and pH value. The 2D-NOESY spectra clearly showed that there were several kinds of cross-peaks from NOE interactions between the protons of phenylbutazone and the protons in interior cavities of both generation 6 and generation 3 PAMAM dendrimers. The solubility, 2D-NOESY results and ITC analysis suggest that encapsulation and electrostatic interaction together caused the solubility enhancement of phenylbutazone. The new techniques such as 2D-NOESY and ITC used in this study are useful tools in investigating the interactions between dendrimers and guest molecules. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:1075–1085, 2009

Keywords: poly(amidoamine); PAMAM; dendrimer; phenylbutazone; solubilization; 2D-NOESY; ITC; interaction; encapsulation; electrostatic attachment

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among one of the most frequently prescribed classes of drugs in the world. They are effective for patients associated with osteoarthritis and other chronic musculoskeletal conditions.[1] A major problem with NSAIDs arises from their extremely low aqueous solubilities, which presents a major challenge during drug formulation.[2–5] Poor solubility of NSAIDs not only restricts their use in topical and parenteral applications, but also causes a low bioavailability and chemical degradation of these drugs in the gastrointestinal tract.[4,5] Up to now, several different strategies were proposed to increase the solubility of

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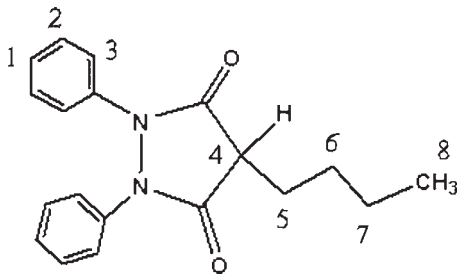
NSAIDs:[6] (1) the micronization of the drug particles;[7] (2) the preparation of water soluble salts of the drugs;[4] (3) the modification of crystal structure of the drugs by formation of polymorphic forms;[8,9] (4) the improvement of the wettability of the drug powder;[10] (5) the addition of solubilizing agents, such as surfactants[11] and (6) the use of soluble and biocompatible drug carriers, such as cyclodextrin,[2] liposomes[12] and dendrimers.[13] During the past decade, the use of drug carriers has proved to be an advisable and promising choice to enhance the solubility of NSAIDs.

Dendrimers are a new class of artificial macromolecules with unique properties, such as nanoscaled globular shapes, well-defined number of reactive functionalities at the periphery, hydrophobic or hydrophilic cavities in the interior and extremely low polydispersity.[14,15] Highly regular branching pattern of dendrimers provides these dendritic architectures well-defined number of periphery functional groups and opportunity for drug molecules to be presented on the surface of dendrimers in a multi-valent fashion.[16–18] In addition, the existence of a large number of relative nonpolar cavities in the interior of dendrimers provides dendrimers the ability of encapsulating drugs in the cores by hydrophobic or hydrogen-bond interactions.[17,19–21] Noncovalent or covalent attachment of these drugs to dendrimers was reported to significantly influence the dissolution rate, the aqueous solubility, the stability, and other physico–chemical properties of the drugs in physiological conditions.[16] Our previous studies have shown that polyamidoamine (PAMAM) dendrimers, the most investigated dendrimers synthesized by D.A. Tomalia and coworkers, are promising nanocarriers for parenteral administration of NSAIDs such as intravenous injection and transdermal delivery.[22] PAMAM dendrimers of different generations have proven to be excellent solubility enhancers,[13] effective sustained release containers[23] and potent transdermal vehicles of NSAIDs[22] based on these studies.



Scheme 1. Chemical structure and proton labeling of phenylbutazone.

In this study, we examined the suitability of PAMAM dendrimers (G3, G4, G4.5, G5, and G6) as drug carriers of phenylbutazone (Scheme 1), a well-established NSAID. The interactions between dendrimers and phenylbutazone were further characterized by two dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) and isothermal titration calorimetry (ITC) techniques. To our best knowledge, there is no reference devoted to the use of PAMAM dendrimers as drug carriers of phenylbutazone at current stage, and 2D-NOESY as well as ITC techniques were firstly employed to study the interaction mechanisms between dendrimers and drug molecules.

EXPERIMENTS

Materials

Generation 3-6 PAMAM dendrimers were purchased from Dendritech Inc. (Midland, MI). Phenylbutazone was obtained from Shangqiu Tiankang Fine Chemical Co. Ltd (Henan, China). Deuterium oxide (D_2O) was purchased from Beijing Chongxi High-Tech Incubator co., Ltd (Beijing, China). All the chemicals were used as received without further purification. Double-distilled water was used in the aqueous solubility studies.

Aqueous Solubility Studies

The aqueous solubility study was performed according to the method proposed by Higuchi and Connors. Excess amount of phenylbutazone (10 mg) was added to a constant volume (500 μL) of each dendrimer solution (0–4 mg/mL of each dendrimer generation dissolved in double-distilled water). The test mixtures in closed tubes were then mechanically shaken for 24 h at room temperature to ensure phenylbutazone reaching saturation in each tube. After the attainment of solubility equilibrium, the contents of the test tubes were centrifuged at 10000 rpm for 5 min. The saturated drug solutions were diluted by 400 or 800 times with double-distilled water, and the phenylbutazone concentrations in each diluted solution were then determined from the absorbance at 264 nm using a Perkin–Elmer UV-Vis spectrophotometer.[24] Since the same concentrations of PAMAM dendrimers give no absorbance