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European Journal of Medicinal Chemistry 40 (2005) 1188–1192
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Preliminary Communication

Dendrimers as Potential Drug Carriers. Part I. Solubilization of Non-Steroidal Anti-Inflammatory Drugs in the Presence of Polyamidoamine Dendrimers

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Received 2 June 2005; accepted 14 June 2005

Available online 08 September 2005

Abstract

The aqueous solubility of non-steroidal anti-inflammatory drugs (NSAIDs) Ketoprofen, Ibuprofen, Diflunisal and Naproxen were measured in the presence of the ethylenediamine (EDA) core polyamidoamine (PAMAM) dendrimers at 37 °C. The effect of concentration and generation of the PAMAM dendrimers has been investigated. Results showed that the solubility of NSAIDs in the PAMAM dendrimer solutions was approximately proportional to dendrimer concentration; the solubility of NSAIDs in higher generation PAMAM solutions was in fact higher that those in lower ones; the order of increased solubility of NSAIDs in PAMAM dendrimers at a constant dendrimer concentration and generation was Naproxen > Ketoprofen > Ibuprofen > Diflunisal. Under suitable conditions PAMAM dendrimers can be highly effective used to enhance the solubility of NSAIDs.

Keywords: Polyamidoamine dendrimers; Guest molecules; Drug carrier; Solubility; NSAIDs

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the world, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal conditions [1]. Also, NSAIDs reduce the risk of and mortality from colon cancer by about half and constitute the prototypical colon cancer chemopreventive agents [2].

However, The use of NSAIDs is limited by their significant toxicity. NSAIDs cause a wide variety of reported adverse events, which include gastrointestinal side effects (such as dyspepsia, gastrointestinal bleeding, and even perforation), renal side effects and some additional side effects (such as hypersensitivity reactions and distinct salicylate intoxication) [3]. Among patients using NSAIDs, up to 4% per year suffer serious gastrointestinal complications. Many studies have shown that NSAIDs increase the risk of peptic ulcer complications by several folds [4]. It is now clear that most NSAIDs can damage the esophagus, stomach, duodenum, small and large intestines, and can impair platelet function systemically, with a consequent increase in bleeding from a variety of GI lesions [5].

The side effects of NSAIDs and their potential toxicity has prompted intensive efforts to identify safer alternatives, which will at least maintain their pharmacological properties. It was reported that patients who switch from one NSAID to another are at high risk of developing peptic ulcer complications [6], suggesting that this strategy may not be an appropriate way to treat gastrointestinal side effects. Also, It was suggested that the use of NSAIDs in parenteral could control these clinical side effects. However, poor solubility of NSAIDs restricts their use in topical and parenteral applications. As poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation [7]. In order to improve the solubility of NSAIDs in water, addition of surface active agents and formation of water

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doi:10.1016/j.ejmech.2005.06.010

soluble salts were carried out and to enhance dissolution and absorption rate, increasing the wettability and micronization of drug particles has often been used to increase the bioavailability of poorly water-soluble NSAIDs [8–10], However methods mentioned above have not always been sufficient to achieve this goal.

Many macromolecular drug delivery systems have been developed to enhance the solubility of NSAIDs and limit their side effects, which promise to be safer than their traditional NSAID counterparts over the years [11–13]. A macromolecular drug delivery system is a complex material in which a drug is attached to a carrier molecule such as a synthetic polymer, antibody, hormone or liposome. As the absorption and distribution of the drug in such a system depended on the properties of the macromolecular carrier, parameters such as site specificity, protection from degradation and minimization of side effects can be altered by modifying the properties of the carrier [14].

Dendrimers are hyperbranced, monodisperse, three-dimensional macromolecules, having defined molecular weight and host-guest entrapment properties. They allow the precise control of size, shape and placement of functional groups and combine typical characteristics of small organic molecules and polymers that result in special physical and chemical properties [15–18]. Accordingly, dendrimers have attracted increasing attention for their applications in many fields. Among them the use of dendrimers as a drug carrier in delivery systems has been of great intreset.

Polyamidoamine (PAMAM) with an ellipsoidal or spheroidal shape is one of the most-studied starburst macromolecules. Due to specific synthesis PAMAM dendrimers have some interesting properties, which distinguish them from classical linear polymers, e.g. PAMAM has a much higher amino group density comparing with conventional macromolecules, a third generation PAMAM prepared from ammonia core has 1.24×10^{-4} amine moieties per unit volume (cubic Angstrom units) in contrast to the 1.58×10^{-6} amine moieties per unit volume of a conventional star polymer [18]; Also, PAMAM Dendrimers possess empty internal cavities and many functional end groups which are responsible for high solubility and reactivity. These specific properties make dendrimers suitable for drug delivery systems [19–21]. Drugs or other molecules can either be attached to dendrimers' end groups or encapsulated in the macromolecule interior [22]. The high density of amino groups and special structure in PAMAM dendrimers may be expected to have potential applications in enhancing the solubility of the low aqueous solubility drugs and as delivery systems for bioactive materials [23]. Drugs bound to dendrimers are at early stages of development and data on them are limited. Here, we focus on using PAMAM dendrimers as potential drug carriers, which are emerging as a promising group of safer and perhaps more effective alternatives to traditional NSAIDs. This study uses PAMAM dendrimers (G2-G4) to investigate the potential of PAMAM dendrimers to increase the solubility of NSAIDs as exemplified by Ketoprofen, Ibuprofen, Diflunisal and Naproxen.

The aim of the present work was (1) to investigate the potential of PAMAM dendrimers as solubility enhancers of NSAIDs; (2) to study effect of molecular size and hydrophobic nature of NSAIDs on their solubility in the presence of PAMAM dendrimers.

2. Experiments

2.1. Materials

Ketoprofen was purchased from Hubei Wuxue Xunda Pharmaceutical Co. (Hubei, China). Ibuprofen and Diflunisal were obtained from Juhua Group Pharmaceutical Factory (Zhejiang, China). Naproxen was a gift from Chetou Pharmaceutical Factory (Zhejiang, China). Ethylenediamine, methyl acrylate, methanol (HPLC grade) were obtained from Shanghai Chemical Co. (Shanghai, China). Double distilled-deionized water was used throughout.

2.2. Synthesis of star polymers

PAMAM dendrimers were synthesized by the following method [18]. Ethylenediamine (10.0 g, 0.166 mol) was dissolved in 100 ml methanol in a 1-liter round-bottomed flask. Methyl acrylate (94.6 g, 0.751 mol) was added at 40 °C and the system stirred for 24 h under nitrogen. Excess methyl acrylate was removed under vacuum at room temperature. A Michael addition between the amine and the acrylate yielded a product bearing four terminal methyl ester groups, defined as the G0.5 PAMAM. Subsequently, ethylenediamine (120 g, 2.00 mol) was dissolved in methanol and added to the G0.5 PAMAM and, after stirring for 48 h under nitrogen and removing excess reactants by vacuum distillation, a product bearing four terminal amino groups were obtained, defined as the G1 PAMAM. By repeating the above cycle, higher generation PAMAM dendrimers (up to G5) were synthesized. For sample preparation, 1-2 wt% PAMAM/ethyl acetate solution was repeatedly filtrated through a 0.1 um nylon filter. Purity of the amine-terminated PAMAM dendrimers were characterized via FT-IR (MAGNA-IR 750, Nicolet Instrument Co., U.S.A), ^{1}H and ^{13}C NMR (DMX-500, German), Mass spectral analysis (BIFI EXTM 3, German) and Element analysis (VARIO EL 3, Elementar Instrument Co., German).

2.3. Solubility testing experiments

Excess NSAIDs drugs was added to 5 ml of each test solution to ensure the drug solution reaching saturation. The solution was mechanically shaken for 24 h at 37 °C and then centrifuged at 5000 rpm for a minute. The absorbances of NSAIDs test solutions at their characteristic wavelengthes (260 nm for Ketoprofen and Naproxen, 221 nm for Ibuprofen and 250 nm for Diflunisal) were tested using the Varian Cary VIII spectrophotometer. Three repeats were conducted.

3. Results and discussion

3.1. Effect of PAMAM concentration and generation on solubility of NSAIDs

The effect of dendrimer concentration on solubility of NSAIDs in the presence of PAMAM dendrimers were measured at 37 °C, and the results were shown in Fig. 1–4. It was observed that the extremely low water solubility of NSAIDs has been significantly improved by PAMAM dendrimers. Take G4 PAMAM dendrimer for an example, after interactions with PAMAM dendrimer at a concentration of 10 mg/ml, Ketoprofen solubility increased from 0.88 up to 16.92 mg/ml; While Naprofen solubility ranged from 0.02 to 31.41 mg/ml, Ibuprofen solubility ranged form 0.10 to 7.45 mg/ml and Diflunisal ranged from 0.19 to 4.94 mg/ml. The apparent solubility of NSAIDs increased linearly as a function of PAMAM dendrimer solution over the concentration range 0-5 mg/ml. At higher concentrations, the solubility of NSAIDs was slightly lower than predicted. This may because of precipitation of an insoluble complex at high concentrations of PAMAM dendrimer. In fact, we observed yellow precipitation at higher concentrations in our experiment. The same behavior was observed for the four selected NSAIDs in all the three generations of PAMAM dendrimer.

The increase of solubility of extremely low water solubility of NSAIDs was presumably contributed to the increase in the number of surface amines and internal cavities that are available to interact with NSAID molecules. Due to the specific and interesting property of PAMAM dendrimers, the cavities in PAMAM dendrimers can keep small guest molecules inside and make dendrimers suitable for enhancing the solubility of drug molecules such as NSAID molecules in aqueous solutions. Also, there are tertiary amines in these internal cavities, which could interact with the atoms of the NSAID molecules by hydrogen bond formation. Furthermore, PAMAM dendrimers have primary amines on the surface, which could interact electrostatically with the carboxyl group in the NSAID molecules. Therefore, PAMAM dendrimers possess open and internal cavities and many func-

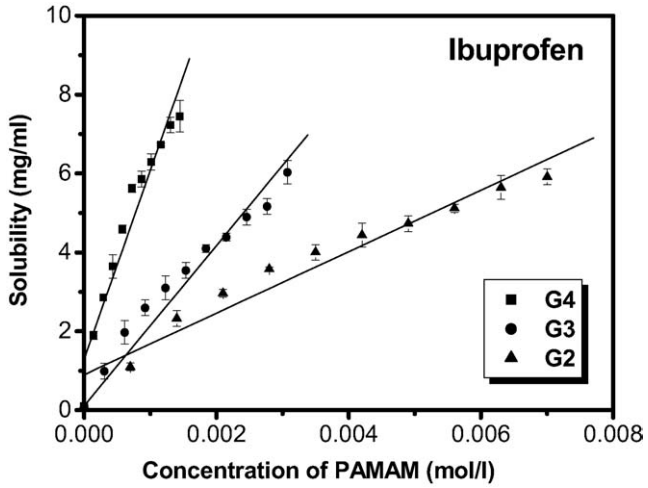


Fig. 1. Solubility of Ibuprofen in the presence of increasing concentration of PAMAM dendrimers.

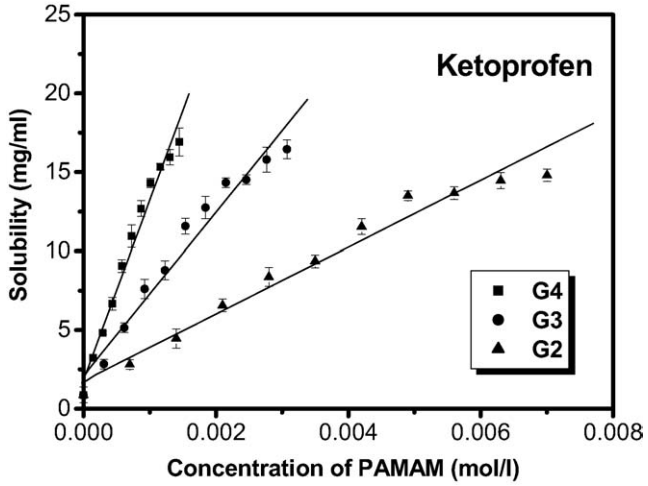


Fig. 2. Solubility of Ketoprofen in the presence of increasing concentration of PAMAM dendrimers.

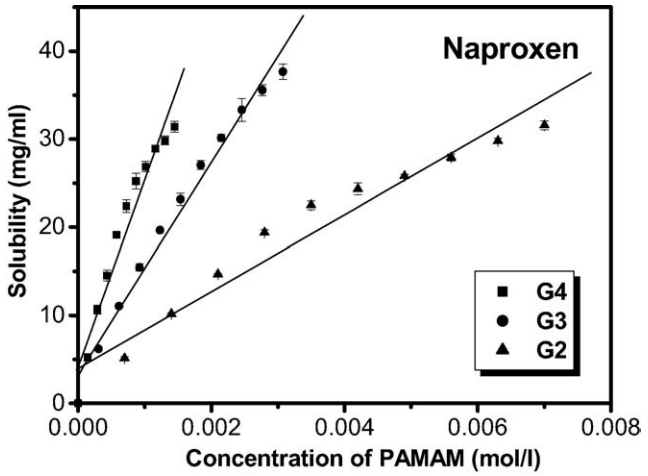


Fig. 3. Solubility of Naproxen in the presence of increasing concentration of PAMAM dendrimers.

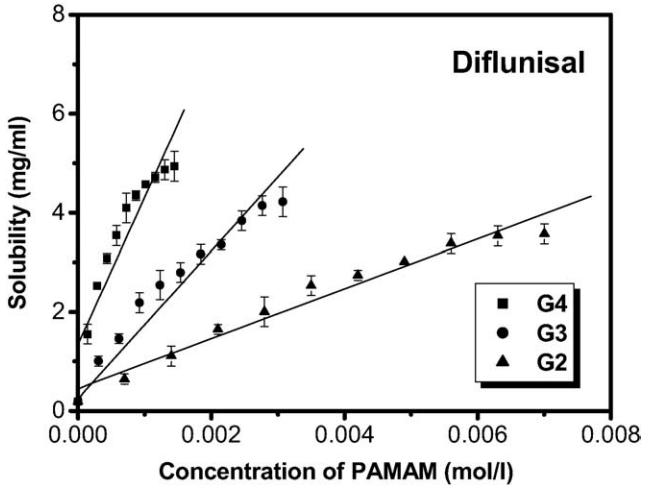
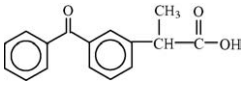
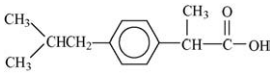
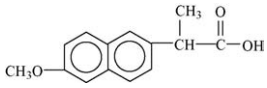
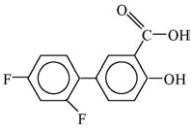


Fig. 4. Solubility of Diflunisal in the presence of increasing concentration of PAMAM dendrimers.

Table 1
The characteristic data of the four NSAIDs in our experiment

Chemical Name	Ketoprofen	Ibuprofen	Naproxen	Diflunisal
Molecular Structure				
Class	Non-Steroidal Anti-Inflammatory Drugs			
Empirical Formula	$C_{16}H_{14}O_3$	$C_{13}H_{18}O_2$	$C_{14}H_{14}O_3$	$C_{13}H_8F_2O_3$
Molecular Wight (g/mol)	254.28	206.28	230.26	250.20
Characteristic Wavelengths (nm)	260	221	260	250
Solubility	Extremely low solubility in water			
PK_a	4.5	5.3	4.2	3.3

tional terminal groups which are responsible for high solubility and reactivity. These specific properties make dendrimers suitable for drug delivery systems [19].

The effect of various generations of PAMAM dendrimers (G2-G4) on the process was investigated at 37 °C. The results are also shown in Fig. 1–4, from which it is clear that the solubility of NSAIDs was affected by the generation of PAMAM dendrimer. The solubility of NSAIDs in higher generation PAMAM solution was in fact higher that those in lower ones at the same concentration. The solubility of hydrophobic compounds in dendrimer solutions likely depends on the dendrimer generation (size) [14]. Since the number of primary and tertiary amines in the dendrimer increases with generation size, at a given pH condition, higher generation dendrimer has a tendency to entrap more hydrophobic compound inside than lower ones. Also, the solubility of NSAIDs in PAMAM solutions depends on the surface area and primary amino groups of PAMAM particles, which cause the higher generation PAMAM particles to have a higher ability to absorb and interact with NSAID molecules. In this way, we could explain why higher generation dendrimers could enhance the solubility of NSAIDs more efficiently than lower ones.

3.2. Effect of molecurlar size and hydrophobic nature of NSAIDs on the their solubility in the presence of PAMAM dendrimers

Experiments were carried out using four familiar NSAIDs, Ketoprofen, Ibuprofen, Diflunisal and Naproxen. Some important physical and chemical properties of these NSAIDs are given in Table 1. The order of increased solubility of NSAIDs in PAMAM dendrimers at a constant dendrimer concentration and generation was Naproxen > Ketoprofen > Ibuprofen > Diflunisal. Take Ketoprofen and Ibuprofen for example, the solubilizing of PAMAM dendrimer was more pronounced for Ketoprofen than for Ibuprofen. The molecular size of the guest, which is similar for Ibuprofen and Ketoprofen, seem not to play a role in the affinity degree for the host. The solubility of Diflunisal in PAMAM solutions was the lowest of the four drugs corresponded to that of the hydrophilic character of the guest molecules. It is interesting that the solubility of Naproxen in the PAMAM dendrimer was increased most significantly in all the four drugs. This adverse result may be deduced to a different interaction mechanism for this molecule. The precise reason for this result will require further investigation.

4. Conclusion

Different generation (G2-G4) PAMAM dendrimers have the potential to significantly enhance the solubility of NSAIDs. The higher solubility may contribute to a higher drug bioavailability. The drug solubility depends on the concentration and the generation of PAMAM dendrimer. Solubility of NSAIDs in the dendrimer solutions increase in an approximately linear manner with an increase in dendrimer concentration; The order of increased solubility of NSAIDs in PAMAM dendrimers at a constant dendrimer concentration and generation was Naproxen > Ketoprofen > Ibuprofen > Diflunisal. Both observations are evidence of the solubility enhancement of NSAIDs is due to an electrostatically interaction between the surface amine groups of dendrimer molecule and the carboxyl group of NSAID and to hydrogen bond formations between tertiary amines in internal cavities of dendrimers and the atoms of NSAID.

Although dendrimer drug-delivery is in its infancy, it offers several attractive features. It provides a uniform platform for drug attachment that has the ability to bind and release drugs through several mechanisms. Our future work will focus on evaluating the relationship between dendrimers and drug molecules. To discuss their advantages and disadvantages in vitro and in vivo release systems. Although toxicity problems may exist, modification of the structure of dendrimers should resolve these issues.

Acknowledgements

Financial supports from Innovation Foundation of Graduate Student in University of Science and Technology of China (KD2004035) were highly appreciated.

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