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Short communication

Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: An in vitro study

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Abstract

Quinolones, an expanding class of clinically established potent antibiotics, is not freely soluble in water which prevents the design of liquid dosage forms and restricts their use in topical applications. In the present study we investigated the potential of polyamidoamine (PAMAM) dendrimers as drug carriers of quinolones (nadifloxacin and prulifloxacin) by aqueous solubility and antibacterial activity studies. Results showed that the aqueous solubility of nadifloxacin and prulifloxacin was significantly increased by PAMAM dendrimers. Microbiology studies showed that nadifloxacin and prulifloxacin still exhibit their strong antimicrobial activities in the presence of dendrimers. These studies indicated that PAMAM dendrimers might be considered as biocompatible carriers of quinolones under suitable conditions.

Keywords: Polyamidoamine dendrimers; PAMAM; Drug carrier; Antibacterial drugs; Quinolones; Nadifloxacin; Prulifloxacin

1. Introduction

Bacterial infections remain major causes of morbidity and mortality in hospitals around the world [1]. A new report estimated that *Staphylococcus aureus* (*S. aureus*) infections alone resulted in 9.5 billion dollars of extra hospital charges and nearly 12,000 inpatient deaths per year [2].

Quinolones, an expanding class of clinically established potent antibiotics, whose accidental discovery occurred in the early 1960s, were very important in addition to the antibiotics that we had already developed [3]. They are well known broad-spectrum antibacterials and widely used to treat numerous diseases [4]. Quinolones cover a host of aerobic gram-negative, gram-positive and even some anaerobic species responsible for various infections (prostatitis, tuberculosis, pneumonia, bronchitis and urinary tract, respiratory tract, skin, gastrointestinal, bone, joint, soft tissue, abdominal infections, some sexually transmitted diseases and some infections that affect people with AIDS). Also, quinolones have gained popularity in the ophthalmology field since they have been shown to be equivalent to combination therapy in the treatment of many ocular infections [3–5].

Quinolones are usually available as tablets or liquid suspension to be taken by mouth.

Although quinolones are well absorbed when orally given, they can still cause side effects. The most common of which involve mainly the digestive system (stomach pain or upset, nausea, vomiting, and diarrhea) [4,5]. In some situations, quinolones should be given by intravenous injection for more serious infections. However, such compounds exist mainly in their zwitterionic form owing to the acid/base interaction

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between the basic nitrogen of the piperazine and the carboxylic acid group. Such interaction also determines the low aqueous solubility of these compounds at pH close to 7 [6–8]. This is the main factor, which prevents the design of liquid dosage forms and restricts their use in topical applications, such as parenteral and ophthalmic, because the aqueous compatibility of these drugs occurs at rather basic or acid pH [9]. Furthermore, poor solubility is generally related to a low bioavailability, which presents a major challenge during drug formulation. In order to improve the solubility of quinolones in water, carbomer hydrogel–quinolone complexes were prepared to enhance dissolution and absorption rate [6]. Also, liposomes were used to encapsulate quinolones [7]. More recently, a new class of water-soluble quinolones was synthesized [10]. However, the aqueous solubility of carbomer hydrogels or liposomes is insufficient to stabilize drugs at high doses. Moreover, high synthesis costs limit the use of these new quinolones.

Dendrimers are new artificial macromolecules topologically based on the structure of a tree. They are hyperbranched, monodisperse, three-dimensional molecules, having defined molecular weight and host–guest entrapment properties. Due to their special synthesis in a stepwise manner from branched monomer units, they allow the precise control of size, shape, dimensions, density, polarity, flexibility, solubility and placement of functional groups by choosing of these building units and functional group chemistry. As a result, they combine typical characteristics of small organic molecules and polymers that result in special physical and chemical properties [11–14]. Up to now, dendrimers have already attracted increasing attention for their applications in many fields including model chemistry or combination chemistry, electrochemistry and photochemistry, nanoparticle synthesis template, water purification, dye decolorization, monomolecular membranes, curing agents in epoxy resin systems, catalyzer in extensive areas, drug delivery systems and gene transfection in biomedical fields. Among them the use of dendrimers as drug carriers in delivery systems has been of great interest.

Polyamidoamine (PAMAM) dendrimer 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 the 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 [14]. The high density of functional groups ($-NH_2$, $-COOH$, $-OH$) in PAMAM dendrimers may be expected to have potential applications in enhancing the solubility of low aqueous solubility drugs and delivery systems for bioactive materials [20]. Also, These functional groups on the outer shell are responsible for high reactivity which means dendrimers can be modified or conjugated with a list of interesting guest molecules. Furthermore, PAMAM dendrimers possess empty internal cavities which are able to encapsulate hydrophobic guest molecules in the macromolecule interior. Drugs or other molecules can either be attached to dendrimers' end groups or encapsulated in the macromolecule interior [19]. These specific properties make dendrimers suitable for drug delivery systems [15–18]. Drugs bound to dendrimers are at early stages of development and data on them are limited. Several authors reported on the encapsulation of non-steroidal anti-inflammatory drugs (NSAIDs) and anti-cancer drugs in dendrimers [21,22]. In the previous study, we also studied the solubilization of sulfonamides by dendrimers [23]. However, to our knowledge there are no studies devoted to the solubilization of quinolone antibacterial drugs in the presence of dendrimers. Here, we focus on using ethylenediamine (EDA) core PAMAM dendrimers as potential drug carriers, which are emerging as a promising group of safer and perhaps more effective alternatives to traditional quinolones as exemplified by nadifloxacin and prulifloxacin.

Nadifloxacin, a fluorinated quinolone antimicrobial, is widely used for the treatment of multiple inflamed acne lesions as a topical agent [23]. It has a potent bactericidal activity against *P. acnes* and other gram-positive and gram-negative bacteria [24]. In addition to its bactericidal activity, nadifloxacin has also been suggested to have anti-inflammatory actions which may have a beneficial effect on some aspects of inflammatory acne [23–25]. Furthermore, nadifloxacin does not show cross-resistance with other new quinolones. Prulifloxacin, the lipophilic prodrug of ulifloxacin, is also a broad-spectrum oral fluoroquinolone antibacterial agent [26]. After oral administration, prulifloxacin is absorbed by the intestine and rapidly metabolized by esterases to ulifloxacin, the active metabolite of prulifloxacin [27–30].

The aim of the present work was (a) to investigate the potential of PAMAM dendrimers as solubility enhancers of quinolones as exemplified by nadifloxacin and prulifloxacin; (b) to study the effect of concentration, generation and pH value on the solubility of nadifloxacin and prulifloxacin and (c) to investigate antibacterial activities of quinolones in the presence of PAMAM dendrimers.

2. Experiments

2.1. Materials

Nadifloxacin and prulifloxacin were purchased from Beijing Maijing Pharmacy Factory (Beijing, China); ethylenediamine, methyl acrylate, methanol, dimethyl sulfoxide (HPLC grade) were obtained from Shanghai Chemical Co. (Shanghai, China). For both solubility and antibacterial studies, distilled water was used.

2.2. Synthesis of PAMAM dendrimers

PAMAM dendrimers were synthesized according to Ref. [31]. Ethylenediamine (10.0 g, 0.166 mol) was dissolved in 100 ml methanol in a 1-l round-bottomed flask. Methyl acrylate (94.6 g, 0.751 mol) was added at 40 °C and the system