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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.
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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

was 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 was obtained, defined as the G1 PAMAM. By repeating the above cycle, higher generation PAMAM dendrimers (up to G5) were synthesized. Purity of the synthesized PAMAM dendrimers was characterized via FT-IR, ^{1}H and ^{13}C NMR and element analysis [14].

2.3. Solubility test

The solubility of nadifloxacin or prulifloxacin was determined using the equilibrium solubility method [32]. Excess drugs were added to 500 μl of each test solution to ensure the drug solution reaching saturation. The solution was mechanically shaken for 24 h at 37 °C and then the solutions were centrifuged at 10,000 rpm for 3 min. Three repeats were conducted for each sample. The effect of initial pH condition on solubility of nadifloxacin and prulifloxacin was examined over the range 7–10, which was achieved by addition of 0.1 M HCl in 10 mg/ml G4 PAMAM dendrimer solutions. The obtained dendrimer solutions with different pH values (7–10) were used for nadifloxacin and prulifloxacin solubility studies as mentioned above.

2.4. UV–vis spectroscopy

Quinolones in ethanol solution or in distilled water give maximum absorbance in UV region at their characteristic wavelengths (297 nm for nadifloxacin and 281 nm for prulifloxacin). Perkin–Elmer UV–vis spectrometer was used to estimate the amount of drug incorporated in the dendrimer. The saturated solutions obtained from the solubility studies were diluted to a proper concentration (500×, distilled water). Since the dendrimers in the diluted solutions give extremely low absorbance between 250 and 700 nm, the absorbance obtained from quinolones dendrimer solution would be solely from nadifloxacin or prulifloxacin. The absorbance of nadifloxacin and prulifloxacin at their characteristic wavelengths was related with the amount/solubility of quinolones in the dendrimer solutions or distilled water.

2.5. Antibacterial activity test

The compounds were tested against *Escherichia coli* (*E. coli*) for their antibacterial activities, using a common Luria–Bertani liquid medium microdilution method as described in Ref. [33]. Before the antibacterial tests, the drug formations in the presence/absence of dendrimer were prepared as follows: nadifloxacin and prulifloxacin were dissolved in 10 mg/ml G4 PAMAM dendrimer to a concentration of 0.5 mg/ml, while pure quinolones were dissolved in DMSO at the same drug concentration. After that, they were diluted in 100 μl of distilled water to reach a final concentration of 5 μg/ml (nadifloxacin or prulifloxacin concentration). Dendrimer and DMSO were also evaluated in the absence of quinolones. When conducting the antibacterial activity studies, 50 μl of Luria–Bertani liquid medium was first distributed from the second to the twelfth well of a 96-well plate. Then, 100 μl of test solutions prepared as above were added to the first test well of each line, and 50 μl of scalar dilution was transferred from the second to the eleventh well, the twelfth well was added without any drug. Finally, 50 μl of a microbial suspension ($\sim 10^{6}$ colony forming units, CFU/ml), obtained from an overnight growth at 37 °C, was added to each well of the plate. The final concentration of these samples used to evaluate the antibacterial activity was from 2.5 μg/ml (second well) to 4.9 ng/ml (eleventh well). The plate was incubated for 24 h at 37 °C and examined by measuring the optical density in a spectrophotometer (630 nm).

3. Results and discussion

3.1. UV–vis spectroscopy studies about the interactions between dendrimers and quinolones

The interactions between quinolones with dendrimer molecules were determined by UV–vis spectroscopy. The UV absorption peak of the free nadifloxacin appeared at 297 nm (Fig. 1). After the addition of G4 PAMAM dendrimers, blue shifts were observed (the peak shifted about 8 nm from 297 nm). In general, blue shift means that drug molecules move to hydrophobic environment, which is an indication of hydrophobic encapsulation of nadifloxacin by the hydrophobic cavities of PAMAM dendrimers. In addition, electrostatic interaction between the carboxyl group of nadifloxacin and the amine groups of PAMAM dendrimers can also explain this blue shift. From the calibration curve of free nadifloxacin in the absence/presence of PAMAM dendrimers, the absorbance of the samples at 297/289 nm showed a linear relationship

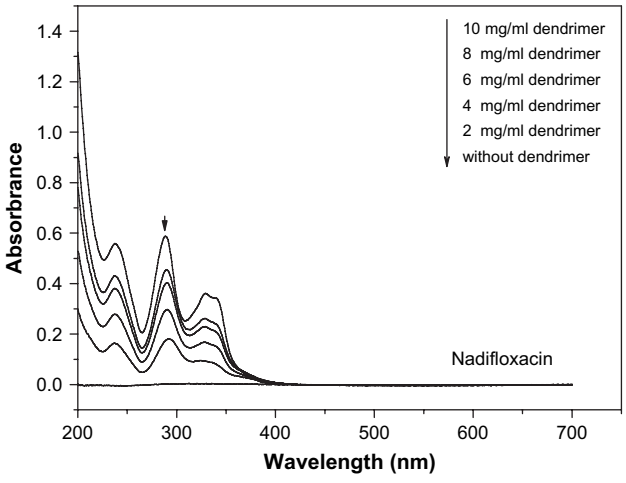

Fig. 1. UV–vis spectrum of nadifloxacin at different G4 PAMAM dendrimer concentrations.

with the concentration of nadifloxacin (data not shown). The absorbance of the test solutions at 289 nm obtained from solubility studies increased significantly with the concentration of PAMAM dendrimers. Since the PAMAM dendrimers in the diluted solutions give nearly no absorbance at 289 nm at the studied concentration, the absorbance obtained from samples would be solely from the drug, which indicated that the solubility of nadifloxacin increased significantly with dendrimer concentration. Similar results were obtained when prulifloxacin were investigated, as shown in Fig. 2, the UV absorption peak of the dendrimer–prulifloxacin mixture appeared at 276 nm, shifting 7 nm from the peak of free prulifloxacin (281 nm). These results concluded that quinolone molecules could interact with PAMAM dendrimers by electrostatic interaction as well as hydrophobic encapsulation.

3.2. Effect of PAMAM dendrimer concentration on solubility of quinolones

The effect of PAMAM dendrimer on solubility of nadifloxacin was carried out using G4 PAMAM dendrimer of molecular weight 6900 Da and 32 amine groups in the outer shell, and the results are shown in Fig. 1. It was observed that the solubility of nadifloxacin increased significantly with PAMAM concentrations. In the presence of G4 PAMAM dendrimer, the solubility of nadifloxacin in the dendrimer solutions increased in an approximately linear manner with an increase in dendrimer concentration. Similar results were obtained with prulifloxacin (Fig. 2). These results were presumably due to the increase in the number of surface amines and internal cavities that are available to interact with or encapsulate nadifloxacin molecules. Firstly, PAMAM dendrimers have large numbers of primary amines on their surface, which could interact electrostatically with the carboxyl group in the quinolone molecules [21,34]. Secondly, PAMAM dendrimers possess empty internal cavities and an open structure (low generation PAMAM dendrimers) [35], due to these specific and interesting properties of PAMAM dendrimers, the hydrophobic cavities in PAMAM dendrimers can keep small guest molecules inside and make dendrimers suitable for enhancing the solubility of hydrophobic drug molecules such as nadifloxacin and prulifloxacin in aqueous solutions [36,37]. Furthermore, there are tertiary amines in these internal cavities, which could interact with the atoms of the quinolone molecules by hydrogen bond formation [37]. Therefore, PAMAM dendrimers possess open and internal cavities and many terminal functional groups which are responsible for high solubility and reactivity. These specific properties make PAMAM series dendrimers suitable for solubility enhancers of quinolones.

3.3. Effect of initial pH condition of dendrimers on solubility of quinolones

To ascertain the most effective initial pH condition on solubility of quinolones using PAMAM dendrimers, dendrimer solutions were produced at a range of pH values (7–10), the concentration of G4 PAMAM dendrimer being constant. The results are shown in Figs. 3 and 4. As can be seen, the process was highly pH-dependent. The solubility of nadifloxacin and prulifloxacin in PAMAM dendrimer solutions was highest at pH 10, less at pH 9 and 8, and least at pH 7. As discussed in Section 3.2, the solubility enhancement of quinolones is due to (a) electrostatical interaction between the surface amine groups of dendrimer molecule and the carboxyl group of quinolones, (b) hydrophobic and open cavities in PAMAM dendrimers and (c) hydrogen bond formations between tertiary amines in internal cavities of dendrimers and the atoms of guest molecules. At lower pH conditions, there is no significant increase of solubility of nadifloxacin in dendrimer solution compared to that at higher pH conditions. This is because the nadifloxacin or prulifloxacin, exist mainly in their zwitterionic form owing to the acid/base interaction between the basic nitrogen of the piperazine and the carboxylic acid group, is unionized at pH conditions close to 7 and hence cannot interact electrostatically with the surface amine groups of dendrimer molecule. Furthermore, earlier studies have shown

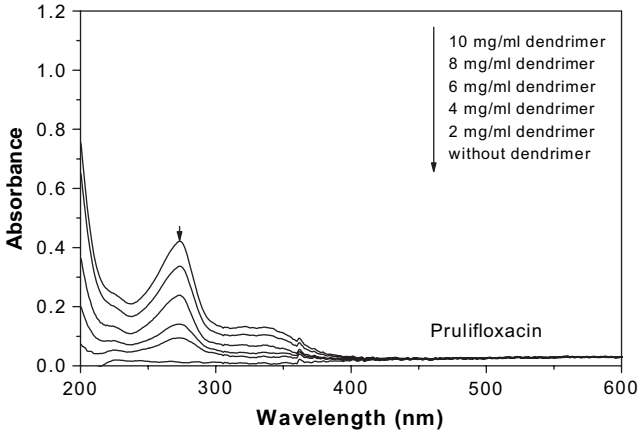

Fig. 2. UV–vis spectrum of prulifloxacin at different G4 PAMAM dendrimer concentrations.

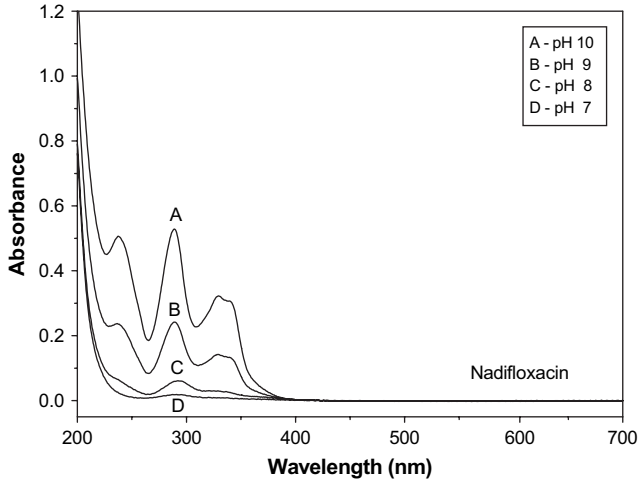

Fig. 3. UV–vis spectrum of nadifloxacin at different pH conditions (G4 dendrimer concentration being constant, 10 mg/ml).

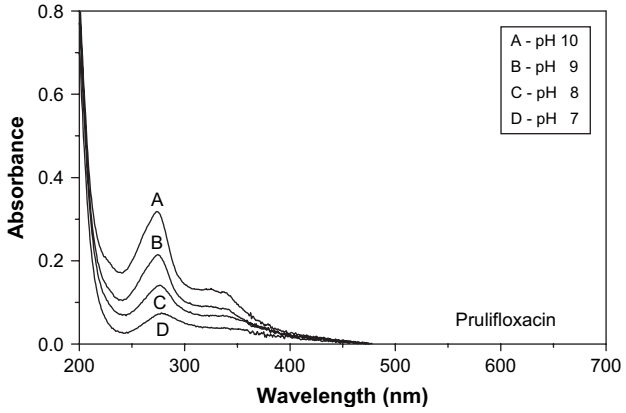

Fig. 4. UV–vis spectrum of prulifloxacin at different pH conditions (G4 dendrimer concentration being constant, 10 mg/ml).

that the cavity environment inside the PAMAM dendrimer is more hydrophobic than that of the water phase outside [38]. Therefore, in PAMAM dendrimer solutions, quinolone molecules could be solubilized in the cavities of the PAMAM dendrimer at the site of low polarity [37]. However, at low pH conditions, protonation of tertiary amines in the cavities of PAMAM dendrimers enhances the polar level of environment inside the dendrimer, which causes no significant increase in the solubility of nadifloxacin and prulifloxacin as observed in the experiment. As a result, the solubility of nadifloxacin in PAMAM dendrimer solutions was lowest at pH 7.

3.4. Effect of PAMAM generation on solubility of quinolones

The effect of various generations of PAMAM dendrimers (G3–G5) in the process was investigated. The results are shown in Figs. 5 and 6, from which it is clear that the solubility of nadifloxacin and prulifloxacin were affected by the generation of PAMAM dendrimer. The solubility of nadifloxacin in higher generation PAMAM solution was in fact higher than those in lower ones. The solubility of hydrophobic compounds in

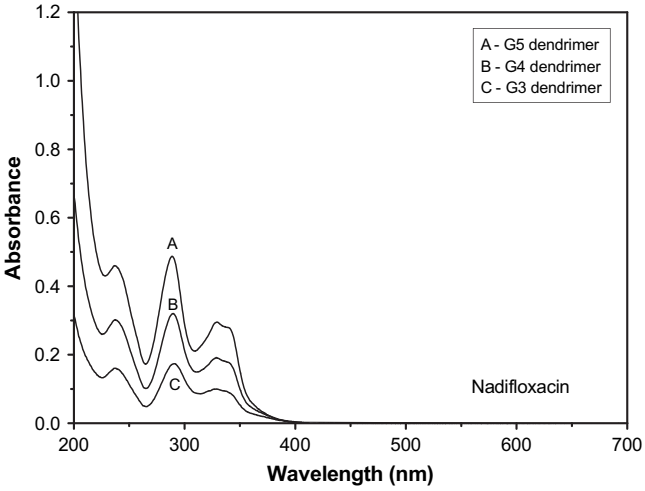

Fig. 5. UV–vis spectrum of nadifloxacin at different PAMAM dendrimer generations (dendrimer mole concentration being constant, 7×10^{-4} M).

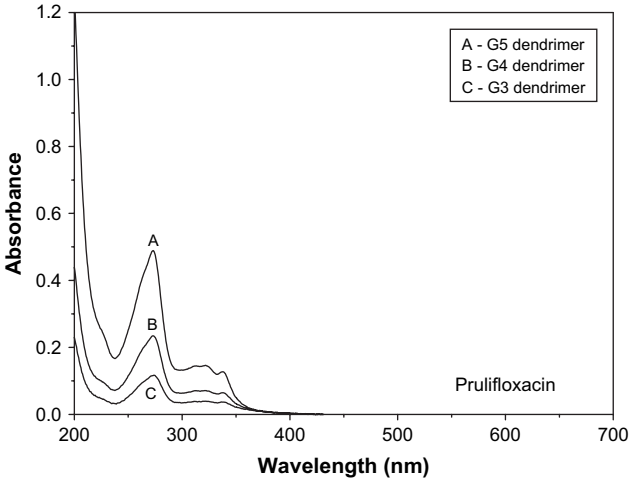

Fig. 6. UV–vis spectrum of prulifloxacin at different PAMAM dendrimer generations (dendrimer mole concentration being constant, 7×10^{-4} M).

dendrimer solutions likely depends on the dendrimer generation (size) [39]. 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 nadifloxacin 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 the nadifloxacin molecule. In this way, we could explain why higher generation dendrimers could enhance the solubility of nadifloxacin more efficiently than lower ones. Similar results were obtained when prulifloxacin were studied.

3.5. Antibacterial activities

To check whether the quinolones still exhibit their antibacterial activities in the presence of PAMAM dendrimers, the antibacterial activity of quinolones (nadifloxacin and prulifloxacin), dendrimer and quinolone–dendrimer solution were investigated. The results presented in Figs. 7 and 8 indicate

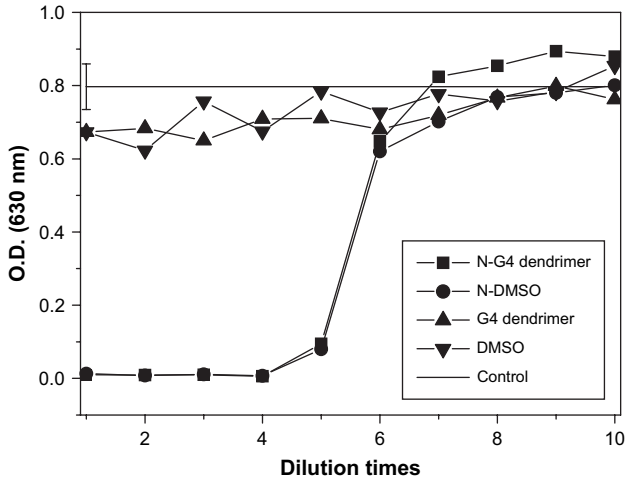

Fig. 7. Antibacterial activity of different formulations of nadifloxacin.

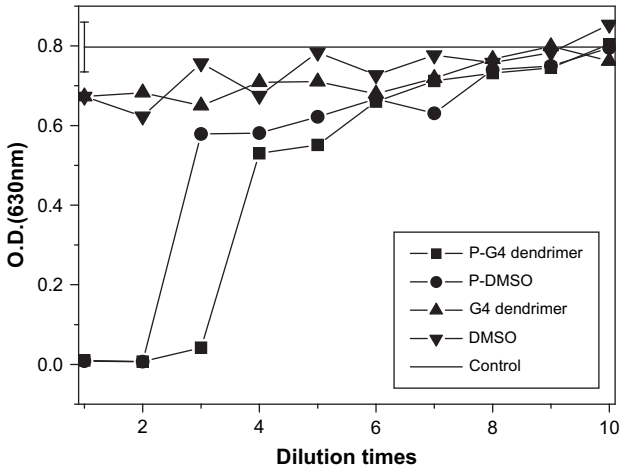

Fig. 8. Antibacterial activity of different formulations of prulifloxacin.

that nadifloxacin and prulifloxacin still display their antibacterial activity against *E. coli* in the presence of PAMAM dendrimers. Interestingly, when equal amounts of free prulifloxacin and prulifloxacin-G4 PAMAM dendrimer are considered (the actual amount of prulifloxacin in the dendrimer solution was equal to the free drug used), prulifloxacin–PAMAM dendrimer is definitely more potent than free prulifloxacin dissolved in DMSO (a 2-fold increase in antibacterial activity). As pure G4 PAMAM dendrimer displayed antibacterial activity against *E. coli* at a much higher concentration (data not shown), the enhanced antibacterial activity should not be contributed to dendrimer itself. It is known that quinolones exert their effects by damaging the bacteria's DNA causing a bactericidal effect. They do this by inhibiting two bacterial enzymes, DNA gyrase and topoisomerase IV, which are involved in bacterial DNA synthesis and replication, thus inhibiting the bacterial multiplication. As PAMAM dendrimers with primary amine surface functional groups could penetrate through cell membrane [40], we could presume that the enhanced antibacterial activity was contributed to the dendrimers which might favor the interaction of the drug with its target or help prulifloxacin with penetration through the bacterial membrane. The precise reason for this increased activity is at present unclear. Although further investigations are necessary in this respect, the in vitro results are very promising as they indicate that appropriate complexation with dendrimer can maintain or increase the effectiveness of quinolones while they were used as antibacterial drugs. Such a development would increase the clinical use of quinolones.

4. Conclusion

Although dendrimer drug delivery is in its infancy, it offers several attractive features [41,42]. It provides a uniform platform for drug attachment that has the ability to bind and release drugs through several mechanisms [43,44]. Our work demonstrated that encapsulation/complexation quinolones into/with dendrimers led to excellent solubility of these drugs and similar antibacterial activity with pure drugs themselves. We are in the process of conducting pre-clinical testing to evaluate the potential of dendrimers as carrier for nadifloxacin/prulifloxacin and other antibacterial drugs. Although toxicity problems may exist, modification of the structure of dendrimers should resolve this issue.

Acknowledgements

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