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Original article

In situ and *in silico* evaluation of amine- and folate-terminated dendrimers as nanocarriers of anesthetics

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ABSTRACT

The search for new nano-systems for targeted biomedical applications and controlled drug release has attracted significant attention in polymer chemistry, pharmaceutics, and biomaterial science. Controlled drug delivery has many advantages over conventional drug administration, such as reduction of side effects, maintaining a stable plasma level concentration and improving the quality of life of patients. In this study, PAMAM G5 dendrimers and PAMAM G5-folic acid conjugates (PAMAM G5-FA) are synthesized and characterized by mass spectrometry (MALDI-MS). Controlled release studies at different pH values show that PAMAM G5-FA is a good candidate as a carrier for tramadol and morphine, while mathematical modeling is conducted, suggesting that the release process is governed by a diffusion mechanism. In addition, using molecular dynamics simulations, we investigate the structural and energetic properties that facilitate the encapsulation of tramadol and morphine by unmodified and functionalized PAMAM-G5 dendrimers at low, neutral and high pH. Our results correlate well with experimental data, confirming that tramadol and morphine may be encapsulated both by functionalized PAMAM dendrimers and unmodified PAMAM. Moreover, the simulations further reveal that hydrogen-bond and electrostatic interactions govern the affinity the dendrimers for both drugs. This information is envisioned to prove useful for the encapsulation of other drugs and for the design of novel functionalized dendrimers.

1. Introduction

Dendrimers are hyperbranched three-dimensional macromolecules with globular or ellipsoidal shapes. Relevant properties include their nanoscale size, the presence of hydrophobic or hydrophilic cavities, a wide variety of possible functionalities at their peripheries, and extremely low polydispersity [1,2]. The structure of the dendrimers exhibits significantly novel and distinct physical, chemical, and biological properties in comparison to traditional linear polymers [2–4]. Dendrimers have attracted considerable attention because of wide and almost limitless variations on their chemical structure. There exist numerous methodologies for their synthesis, and their unique structures show characteristic properties, which render them a reliable alternative to traditional polymers in a wide range of applications. One alternative is, the design of new nanocontainers and nanodevices for biomedical applications [1,5], such as drug delivery systems [6,7], antiviral agents, and magnetic resonance imaging contrast agents [8,9]. Among the most frequently used dendrimers in biomedical applications are the poly(amidoamine) (PAMAM) dendrimers, which were first synthesized by Tomalia in 1985, being the most thoroughly investigated and characterized as well as the easiest to obtain commercially [1].

PAMAM dendrimers with different surface functionalities have the ability to encapsulate a wide variety of guest molecules for the purpose of drug delivery. It has been reported that PAMAM dendrimers could be efficient delivery systems with the benefits of enhanced drug solubility, prevention of drug

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degradation, increased circulation time, sustained/controlled drug release and potential drug targeting [10]. In addition, the advances in dendrimer surface engineering, i.e. the conjugation of functional groups to the chain ends of dendrimer surface, could provide stimuli-responsive properties to PAMAM dendritic delivery systems, which could add value to drug delivery efficiency and therapeutic efficacy [11].

The functional groups of amine-terminated PAMAM bind a ligand through hydrogen bonds, electrostatic interactions, and hydrophobic interactions [12–14]. In this study, we examined the interactions between amine- and folate-terminated PAMAM dendrimers of fifth generation and pain relief drug such as morphine and tramadol (Fig. 1). PAMAM dendrimers modified with folic acid (FA) via covalent conjugation neutralize the remaining amines of the dendrimers surfaces, decreases the toxicity of the dendrimers and encapsulate varius drug for targeted therapy [15]. For morphine and tramadol, the conventional pharmaceutical formulations must be administered every four and eight hours, respectively, a frequency which often compromises patient compliance. A modified release formulation would increase the dosage interval and thus reduce fluctuations in circulating concentrations of the drug.

In the present study, folate-terminated PAMAM dendrimers of generation five (PAMAM G5-FA) were prepared by simple chemical modification of PAMAM G5 and evaluated for controlled drug delivery of the common pain relief drugs morphine and tramadol. The driving force that controls the drug release behavior was analyzed by molecular simulation techniques in terms of the structure and energetics of each one of the complexes.

2. Results and discussion

2.1. Polyamine-conjugation of the dendrimers PAMAM G5 with folic acid

The γ-carboxylic acid group of folic acid was covalently conjugated to the free surface amine groups of PAMAM G5 through a carbodiimide mediated amide linkage. A higher molar ratio of 1:140 was used to get all the 128-amine groups of the PAMAM G5 dendrimer conjugated. The conjugates were characterized using mass spectrometry and the spectra shows that only 23 folate molecules were attached to the PAMAM G5 dendrimer (Fig. 2) [16]. The amount of folate was also determined by ^{1}H NMR experiments that showed around 20 units attached to the PAMAM G5 (See Fig. SI4–6 of Supporting information). The amount of molecules coupled to PAMAM G5 likely is due to steric hindrance caused by the size of folic acid.

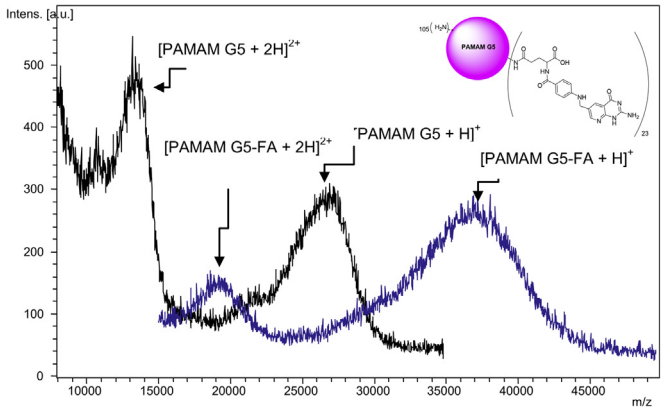

Fig. 2. MALDI-TOF spectra of PAMAM G5 (black) and PAMAM G5-FA (blue) nanocompounds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Drug loading into dendrimer conjugates

To determine the capacity of capture of morphine and tramadol on PAMAM G5 and PAMAM G5-FA, an excess of the pharmacological molecule was reacted with a quantity known of dendrimer. Then, by diffusion analysis across a dialysis membrane and UV spectrophotometry analyses at previously determined wavelengths (285 nm for morphine and 270 nm for tramadol), we obtained an indirect estimate of the moles of drug molecules encapsulated by

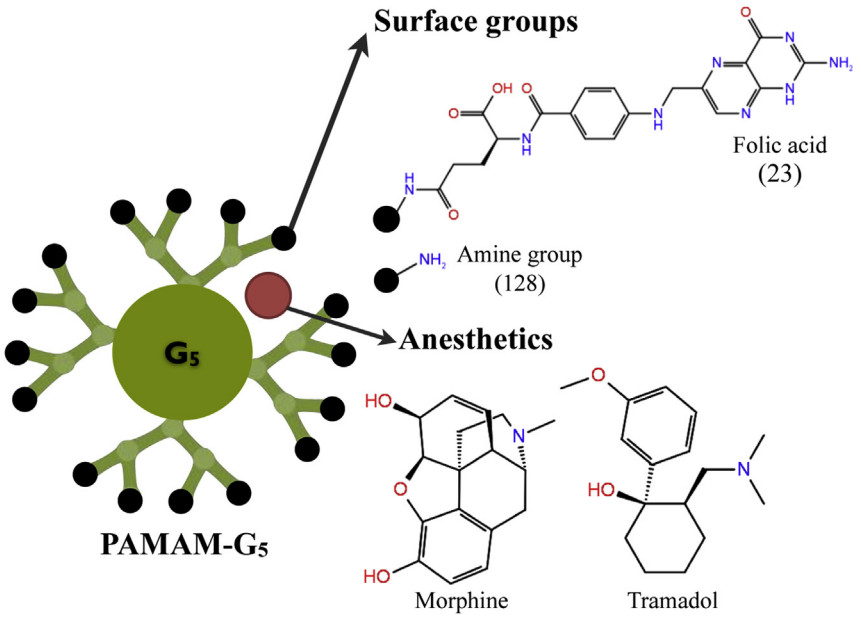

Fig. 1. Schematic representation of amine- and folate-terminated PAMAM dendrimer of generation five and its complexation with the anesthetics: morphine and tramadol. The chemical structures of the surface groups and the drugs are shown at right.