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Research Paper

Construction of 6-thioguanine and 6-mercaptopurine carriers based on βcyclodextrins and gold nanoparticles

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ABSTRACT

As a novel strategy to overcome some of the therapeutic disadvantages of 6-thioguanine (TG) and 6-mercaptopurine (MP), we propose the inclusion of these drugs in βcyclodextrin (βCD) to form the complexes βCD-TG and βCD-MP, followed by subsequent interaction with gold nanoparticles (AuNPs), generating the ternary systems: βCD-TG-AuNPs and βCD-MP-AuNPs. This modification increased their solubility and improved their stability, betting by a site-specific transport due to their nanometric dimensions, among other advantages.

The formation of the complexes was confirmed using powder X-ray diffraction, thermogravimetric analysis and one and two-dimensional NMR. A theoretical study using DFT and molecular modelling was conducted to obtain the more stable tautomeric species of TG and MP in solution and confirm the proposed inclusion geometries. The deposition of AuNPs onto βCD-TG and βCD-MP *via* sputtering was confirmed by UV–vis spectroscopy. Subsequently, the ternary systems were characterized by TEM, FE-SEM and EDX to directly observe the deposited AuNPs and evaluate their sizes, size dispersion, and composition. Finally, the *in vitro* permeability of the ternary systems was studied using parallel artificial membrane permeability assay (PAMPA).

1. Introduction

The drugs 6-thioguanine (TG) and 6-mercaptopurine (MP) are analogues of guanine and adenine (see their structures in Fig. 1a and b) and are used clinically to treat acute lymphoblastic leukaemia (ALL) in children and other types of leukaemia (Erb, Harms, & Janka-Schaub, 1998). In addition, TG is used as an immunosuppressant in transplant surgery, and it has been shown to hinder HIV replication (Krynetskaia et al., 2001). MP is also used for Crohn's disease or together with methotrexate for leukaemia (Chande, Townsend, Parker, & MacDonald, 2016; Schmiegelow, Nielsen, Frandsen, & Nersting, 2014). The applications of TG and MP were discovered in 1951 by Gertrude B. Elion, who reported evidence that they could produce complete remission in children with ALL. These medications were approved by the Food and Drug Administration (FDA) just two years after the first synthesis (Elion, 2008). Despite their proven efficacy as antineoplastic agents, TG and MP have some therapeutic disadvantages. After oral administration, they exhibit poor absorption, reaching sufficient plasma concentrations after 10–12 h. Moreover, food can reduce their bioavailability to a considerable degree. These drugs are distributed throughout the body, preferably focusing on the bone marrow; they do not cross the blood-brain barrier, are widely metabolized in the liver and are excreted through the urine. Several research efforts have been directed towards modifying their structures and synthesizing new nucleoside analogues to promote more selective effects and increase these drugs' aqueous solubility (Bohon & De los Santos, 2003; Erb et al., 1998).

An interesting way to change the physicochemical properties of a drug is through subtle interactions that do not alter its chemical structure. This can be achieved *via* inclusion in cyclodextrin (CD) matrices, leading to new therapeutic approaches (Ceborska, 2014; Tiwari, Tiwari, & Rai, 2010; Zhang & Ma, 2013), even in cancer (Gidwani & Vyas, 2015). βcyclodextrin (βCD) is a cyclic oligosaccharide, is non-toxic, and consists of 7 glucose units linked by α(1–4) bonds, forming a truncated cone (see the structures in

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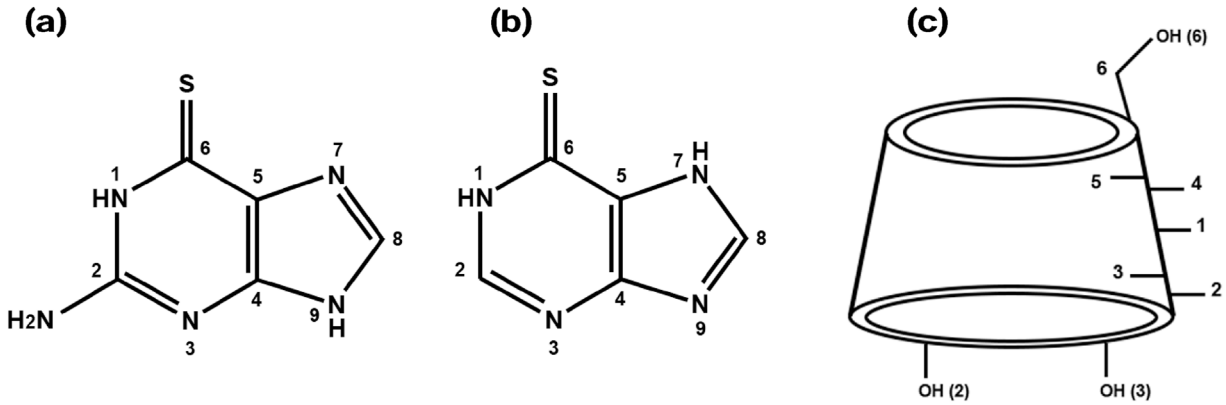

Fig. 1. Structures of (a) TG, (b) MP and (c) βCD with their respective proton assignments.

Fig. 1c) with a cavity diameter of 6–6.5 Å (Del Valle, 2004). Its inner cavity is partially hydrophobic, and its outer surface is hydrophilic. Thus, βCD is capable of including preferably non polar compounds, such as aliphatic chains, aromatic or heterocyclic compounds (Chen, Chang, & Gilson, 2004; Chen, Diao, & Zhang, 2006; Douhal, 2004; Makedonopoulou & Mavridis, 2000; Sierpe et al., 2015; Whang, Vendeix, Gracz, Gadsby, & Tonelli, 2007). βCD has been described in numerous research papers, patents and conference presentations, although most studies have focused on its pharmaceutical applications (Loftsson & Brewster, 2010). As a matrix, βCD has been widely used for drug delivery as it may change some unfavourable properties of included guest molecules. βCD protects drugs from biodegradation; exhibits high thermal stability; with a decomposition temperature close to 300 °C; is stable at basic and neutral pH; prevents the instability of molecules upon exposure to oxygen, water, radiation, heat or internal chemical reactions; reduces irritation caused by the direct entry of some medications to the body; and avoids incompatibility between drugs with other inactive ingredients (Del Valle, 2004; Tiwari et al., 2010). Indeed, the inclusion process may decrease the toxicity and increase the water solubility of drugs. Therefore, inclusions can be used to administer drugs to the body, transport them to the site of action of stably, and release them uniformly over long periods (Loftsson, Vogensen, Brewster, & Konrádsdóttir, 2007; Laza-Knoerr, 2010).

In terms of drug delivery, several works have addressed the inclusion processes of drugs in different CDs (Challa, Ahuja, Ali, & Khar, 2005; Priotti et al., 2015), but the main research interest has focused on increasing their solubility (Crestani, Azevedo, Veiga, & Gomez, 2011; Loftsson & Brewster, 1996). In comparison, little research has been devoted to the relationship and interplay between the solubility and the permeability, which must be associated in order to maximize the overall absorption (Dahan & Miller, 2012). Besides this, only few works has focused on structural features of the guest in the matrix that allow new uses of these CDs, such as stabilizing agents of metal nanoparticles (NPs) or others (Barrientos, Allende, Orellana, & Jara, 2012; Jullian et al., 2015; Monteiro et al., 2017; Sierpe et al., 2015; Vasconcelos et al., 2016). However, studying the guest conformations, possible conformations present, and inclusion geometries may facilitate discussing the values of the association constants, assessing their stability or even elucidating the drug release mechanisms. It would, therefore, be interesting to examine the chemical equilibrium criteria of the inclusion complexes (ICs) and their degree of dissociation in solution. Indeed, this information would allow the interaction force between the matrix and the guest to be estimated. For example, for various organic molecules, theoretical and experimental studies have predicted that the functional groups of the guests may remain exposed (Barrientos, Yutronic, Muñoz, Silva, & Jara, 2009; Sierpe et al., 2015). Studies performed by our research group on the inclusion geometry in complexes using CDs have demonstrated that the presence of gold nanoparticles (AuNPs) can produce a partial displacement of the guest out of the matrix because of the interaction of the guests' functional groups with the metal surface, while the non polar region remains included. Considering possible drug delivery applications, this phenomenon favours the controlled release of drugs during laser irradiation of the AuNPs (Sierpe et al., 2015).

AuNPs can be used to improve drug release and can be easily functionalized with diverse CD-based complexes for potential biomedical applications, including cancer treatments (Heo et al., 2012; Park et al., 2009; Shi, Goodisman, & Dabrowiak, 2013). In general, NPs offer strategies for more specific therapies; their small sizes allow them to penetrate cell membranes, improve targeting and increase the local concentration (De Jong & Borm, 2008). Among the widely diverse metal NPs, research into AuNPs has grown steadily because of their broad spectrum of bioapplications. For example, gold nanospheres with sizes between 4 and 100 nm have been shown to have low cytotoxicity (Connor, Mwamuka, Gole, Murphy, & Wyatt, 2005; Pan et al., 2007; Tirelli, 2006; Yen, Hsu, & Tsai, 2009). Additionally, they can act as nano-vehicles to transport and then release drugs in a sustained and controlled manner (Bansal et al., 2005; Dykman & Khlebtsov, 2012; Sonavane, Tomoda, & Makino, 2008). AuNPs also have notable optical properties; for example, they can absorb and release energy in a localized manner due to the resonant oscillations of free electrons in the conduction band. This process is called the photothermal effect and may be used as a strategy to destroy cancer cells, while releasing drug molecules, thereby providing additional therapeutic benefit (Bayazitoglu, Kheradmand, & Tullius, 2013; Choi et al., 2011; Guerrero et al., 2014; Jing-Liang and Gu, 2010). NPs accumulate preferentially in tumour sites due to the nature of these cells, enabled by the so-called enhanced permeability and retention (EPR effect). Structures of certain sizes tend to accumulate in tumour tissues substantially more than they do in normal tissues due to an increase in the production of blood vessels, extravasations and the lack of effective lymphatic drainage, among other factors. Therefore, biological processes can be influenced by nanometre-scale materials, which implies the potential for advances in the diagnosis and treatment of common diseases, such as cancer (Bertrand, Wu, Xu, Kamaly, & Farokhzad, 2014; Kobayashi, Watanabe, & Choyke, 2014; Nehoff, Parayath, Domanovitch, Taurin, & Greish, 2014).

The aim of this work is to construct a ternary system based on βCD and AuNPs that may be useful as a nanocarrier for different drugs, especially antineoplastic agents, which are poorly soluble, unstable and possess various therapeutic disadvantages, such as TG and MP. To this end, the complete characterization of the complexes obtained, the inclusion geometry and their interaction with AuNPs for potential application in drug release was achieved.

2. Materials and methods

2.1. Reagents and solvents

βCD hydrate (98% purity, 1134.98 g/mol), TG (> 98% purity, 167.2 g/mol) and MP monohydrate (> 98% purity, 170.2 g/mol) were