European Journal of Pharmaceutics and Biopharmaceutics 92 (2015) 120–129

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Release kinetics of paclitaxel and cisplatin from two and three layered gold nanoparticles

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ARTICLE INFO

Article history:
Received 13 December 2014
Revised 13 February 2015
Accepted in revised form 16 February 2015
Available online 7 March 2015

Keywords:
Nanotherapy
Cisplatin
Paclitaxel
Drug release kinetics
Drug release modeling

ABSTRACT

Gold nanoparticles functionalized with biologically compatible layers may achieve stable drug release while avoiding adverse effects in cancer treatment. We study cisplatin and paclitaxel release from gold cores functionalized with hexadecanethiol (TL) and phosphatidylcholine (PC) to form two-layer nanoparticles, or TL, PC, and high density lipoprotein (HDL) to form three-layer nanoparticles. Drug release was monitored for 14 days to assess long term effects of the core surface modifications on release kinetics. Release profiles were fitted to previously developed kinetic models to differentiate possible release mechanisms. The hydrophilic drug (cisplatin) showed an initial (5-h) burst, followed by a steady release over 14 days. The hydrophobic drug (paclitaxel) showed a steady release over the same time period. Two layer nanoparticles released 64.0 ± 2.5% of cisplatin and 22.3 ± 1.5% of paclitaxel, while three layer nanoparticles released the entire encapsulated drug. The Korsmeyer–Peppas model best described each release scenario, while the simplified Higuchi model also adequately described paclitaxel release from the two layer formulation. We conclude that functionalization of gold nanoparticles with a combination of TL and PC may help to modulate both hydrophilic and hydrophobic drug release kinetics, while the addition of HDL may enhance long term release of hydrophobic drug.

1. Introduction

Tumor chemotherapeutic response can be significantly affected by drug physiochemical properties, such as water solubility and bioavailability, as well as intrinsic and physiologic resistance by the tumor tissue itself. Two commonly utilized chemotherapeutics in cancer treatment are cisplatin and paclitaxel [1]. Cisplatin inhibits cell proliferation through multiple mechanisms, including: binding with DNA to form intra-stand adducts causing changes in DNA conformation, promoting mitochondrial damage leading to diminished energy production, altering cellular transport mechanisms, and decreasing ATPase activity within the cells [2,3]. Paclitaxel enhances tubulin polymerization to stable microtubules and stabilizes them against depolymerization, which results in mitotic arrest [4]. While both drugs are effective, they are known to possess adverse reaction profiles. Cisplatin induces renal toxicity caused by its activation within proximal and distal tubules, neurotoxicity by damaging Schwann cells of the myelin sheath, and tumor lysis syndrome (TLS) which results in abnormal metabolic and electrolyte profiles [2]. Paclitaxel has shown dose-limiting hematological toxicity (e.g. neutropenia) and sensory neurotoxicity, along with other adverse non-hematological toxicities including arthralgia, myalgia, and fluid retention [5]. In addition to adverse profiles, the poor water solubility and low bioavailability of paclitaxel have hampered its clinical use. The

Abbreviations: DLS, dynamic light scattering; FTIR, Fourier transform infrared; HDL, high density lipoprotein; HPLC, High Performance Liquid Chromatography; NSCLC, non-small cell lung cancer; PBS, phosphate buffered saline; PC, L-α-phosphatidylcholine; PEG, poly (ethylene glycol); RES, Reticulo-endothelial System; SEM, scanning electron microscope; TFA, Trifluoroacetic acid; TL, 1-hexadecanethiol; UV–Vis, ultraviolet–visible.

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http://dx.doi.org/10.1016/j.ejpb.2015.02.017
0939-6411/

drug is administered in a solubilized form, Cremophor EL, to overcome minimal water solubility; while the castor oil used to solubilize the drug enhances bioavailability, it is known to induce histamine release resulting in hypersensitivity reactions in some patients [6,7].

In order to enhance tumor response while minimizing systemic toxicity, a variety of drugs have been encapsulated in organic or inorganic nanoparticles, ranging in size from 1 to 100 nm. The rate of drug release is dependent upon the physiochemical properties of the drug, attachment strength between drug molecules and the nanoparticle surface, and surface modifications used in the synthesis process. Gold nanoparticles, in particular, have been utilized as agents for drug delivery as well as in thermal therapy, *in vivo* imaging, and in radio-sensitization for both pre-clinical and clinical purposes [8]. Through nanoparticle functionalization, drug release may be modulated to ensure sufficient time for nanoparticles to localize in the tumor or to release drug at specific locations (e.g., hypoxic regions) within the tumor microenvironment [9]. For example, the addition of surfactant poly-(ethylene)-glycol (PEG) is known to escalate nanoparticle circulation time by one to two orders of magnitude compared to freely circulating drugs [10], providing additional time for nanoparticles to localize in the solid tumor tissue. Surface modifications must also ensure that nanoparticles can successfully travel throughout systemic circulation to the tumor, extravasate from the intratumoral capillaries, and diffuse throughout the tissue to reach malignant cells [11]. This can be a challenge as nanoparticles administered *in vivo* are often sequestered and removed from systemic circulation by the reticuloendothelial system (RES) [12].

The heterogeneous cell cycling patterns typically found in tumors ideally require nanoparticle accumulation with a sustained drug release. Paclitaxel-loaded gold nanoparticles have been utilized with this goal in mind while aiming for decreased toxicity and lowered chemoresistance [13,14]. Studies have shown that highly stable PEG-coated gold nanoparticles exhibit a biphasic paclitaxel release pattern with an initial burst followed by a slower release over the next 120 h [15]. Cisplatin-loaded gold nanoparticles show similar release patterns [16–23]. "Smart-sensing" pH-sensitive nanoparticles have been developed that release cisplatin in specific environments, such as the acidic microenvironment of the tumor or within the cellular endosome once cellular internalization has occurred [23]. Recently, controlled release of cisplatin from magnetic nanoparticles has also been evaluated [24,25].

In this study, we examine the release profiles of cisplatin and paclitaxel from novel two and three layer gold nanoparticles for the purpose of aiding the development of gold-based nanotherapeutics [26]. Two layer gold nanoparticles were synthesized by adding hexadecanethiol (TL) and phosphatidylcholine (PC) to the outside of gold cores. The addition of PC to the outer layer of TL creates a hydrophobic region, similar to the lipid bilayer found on liposomes, which can be utilized for loading hydrophobic drugs. For the three layer gold nanoparticles, high-density lipoprotein (HDL) was added to the two layer nanoparticles for the purpose of improving tumor and liver targeting. For both two and three layer gold nanoparticles, paclitaxel was loaded in the hydrophobic region between the TL and PC. Cisplatin was loaded through non-covalent interactions onto the outside of the two or three layer gold nanoparticles. The release of drug was assessed based on particle surface modifications and drug physiochemical properties. Mechanisms of drug release were further assessed by evaluation of kinetic models, including: zero-order kinetic model, first-order kinetic model, simplified Higuchi model, and Korsmeyer–Peppas model [27]. Finally, an assessment of nanoparticle efficacy was performed in 3D cell culture.

2. Materials and methods

2.1. Materials

$HAuCl_4$ (Alfa Aesar, Ward Hill, MA, USA), trisodium citrate (Fisher Scientific, Waltham, MA, USA), 1-Hexadecanethiol (TL) (Sigma Aldrich), 100% Ethanol (Decon Labs, King of Prussia, PA, USA), Chloroform (Sigma Aldrich), L-phosphatidylcholine (PC) (Sigma Aldrich), high density lipoprotein (HDL) (Lee Biosolutions, St. Louis, MO, USA), Phosphate-Buffered Saline (PBS) (Life Technologies, Grand Island, NY), Cisplatin (Sigma Aldrich), Paclitaxel (Cayman Chemical, Ann Arbor, MI, USA), Acetonitrile (Sigma Aldrich), and Trifluoroacetic acid (TFA) (Sigma Aldrich) were purchased.

2.2. Synthesis of citrate gold nanoparticles

Particles were synthesized using a method in which gold chloroauric acid is reduced by trisodium citrate as previously described [28]. In this process, 2.2–2.4 mL 1% weight/volume (wt/v) citrate is added to 200 mL of boiling 0.01% wt/v $HAuCl_4$, and the solution is allowed to continue boiling for 10 min to promote the reaction of sodium citrate to citric acid. Once the reaction is completed, the solution cools at room temperature before concentration using a rotovapor (Buchi Rotovapor System, BÜCHI Labortechnik AG, Flawil, Switzerland) to ~20 mL at 20 OD. After the nanoparticles are concentrated, surface modifications are added as described below.

2.3. Particle functionalization with PC and HDL

The first layer applied to the citrate gold nanoparticles was 1-Hexadecanethiol dissolved in ethanol. Previous studies have shown that thiol compounds can displace surface-bound citrate from gold nanoparticles due to the strong binding affinity between gold and thiol in comparison with the electrostatic binding with citrate [29–31]; a comprehensive review concerning the covalent interaction between gold and sulfur was recently published [31]. This creates a hydrophobic nanoparticle, as the hydrocarbon chains of the thiol compound will point outward from the gold core. While stirring, 20 mL pure ethanol was placed in a beaker with 60 μL 1-Hexadecanethiol being added secondly to reach a molar ratio between thiol and gold nanoparticles of 2500: 1. The 1-Hexadecanethiol solution was added slowly to the nanoparticle solution over the next 10 min, while also undergoing sonication. The sample was further sonicated for two hours, and then placed for 12 h on an orbital rocker (Boekel Scientific, Feasterville, PA, USA). The sample was spun down, and the pellet was washed twice with ethanol and sonicated before suspension in chloroform. The second functionalization was the addition of the PC to the surface of nanoparticles. The stock solution was made by diluting PC in chloroform (2 mg/mL), and 100 μL (molar ratio 2000 PC: 1 NP) was added to the particles after the TL layer, and allowed to set overnight on an orbital rocker. The solutions were transferred to glass tubes and the chloroform evaporated at ambient temperature. This process completed the two layer gold nanoparticles containing gold core, TL, and PC. The three-layered nanoparticles were created by optimizing the ratio of HDL to particle optical density (1 mg HDL per 20 OD nanoparticle), and allowed to react overnight after two hours of sonication.

2.4. Addition of chemotherapeutics to nanoparticles

The amount of chemotherapeutic loaded was chosen to achieve a molar concentration upon release typical for cell culture