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Prediction of nanoparticle prodrug metabolism by pharmacokinetic modeling of biliary excretion

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ABSTRACT

Pharmacokinetic modeling and simulation is a powerful tool for the prediction of drug concentrations in the absence of analytical techniques that allow for direct quantification. The present study applied this modeling approach to determine active drug release from a nanoparticle prodrug formulation. A comparative pharmacokinetic study of a nanoscale micellar docetaxel (DTX) prodrug, Procet 8, and commercial DTX formulation, Taxotere, was conducted in bile duct cannulated rats. The nanoscale (~40 nm) size of the Procet 8 formulation resulted in confinement within the plasma space and high prodrug plasma concentrations. *Ex vivo* prodrug hydrolysis during plasma sample preparation resulted in unacceptable error that precluded direct measurement of DTX concentrations. Pharmacokinetic modeling of Taxotere and Procet 8 plasma concentrations, and their associated biliary metabolites, allowed for prediction of the DTX concentration profile and DTX bioavailability, and thereby evaluation of Procet 8 metabolism.

Procet 8 plasma decay and *in vitro* plasma hydrolytic rates were identical, suggesting that systemic clearance of the prodrug was primarily metabolic. The Procet 8 and Taxotere plasma profiles, and associated docetaxel hydroxy-tert-butyl carbamate (HDTX) metabolite biliary excretion, were best fit by a two compartment model, with both linear and non-linear DTX clearance, and first order Procet 8 hydrolysis. The model estimated HDTX clearance rate agreed with *in vitro* literature values, supporting the predictability of the proposed model. Model simulation at the 10 mg DTX equivalent/kg dose level predicted DTX formation rate-limited kinetics and a peak plasma DTX concentration of 39 ng/mL at 4 h for Procet 8, in comparison to 2826 ng/mL for Taxotere. As a result of nonlinear DTX clearance, the DTX AUCinf for the Procet 8 formulation was predicted to be 2.6 times lower than Taxotere (775 *vs.* 2017 h × ng/mL, respectively), resulting in an absolute bioavailability estimate of 38%. As DTX clearance in man is considered linear, this low bioavailability is likely species-dependent. These data support the use of pharmacokinetic modeling and simulation in cases of complex formulations, where analytical methods for direct measurement of free (released) drug concentrations are unavailable. Uses of such models may include interpretation of preclinical toxicology studies, selection of first in man dosing regimens, and PK/PD model development.

1. Introduction

Liposomes have been utilized for over 30 years to enhance the plasma circulation lifetimes and increase target accumulation of many hydrophilic agents but have provided only modest improvements for hydrophobic drugs such as the taxanes and non-amphipathic camptothecins. Attempts to increase the circulation lifetime of these hydrophobic drugs have included the use of water-soluble polymers such as polyethylene glycol (PEG) [1–3], polypeptides [4–6] and polylactides [7]. Recently, docetaxel was formulated in a targeted nanoparticle formulation composed of PLA-PEG [8]. Although this formulation dramatically increased plasma docetaxel C_{max} and AUC values relative to the equivalent dose of free drug, approximately 99% of the injected dose was cleared from the plasma 24 h after injection to rats.

As an alternative to enhancing plasma levels of docetaxel through formulation of the parent drug, we report here on a prodrug delivery platform that is focused on decreasing non-specific systemic drug exposure and enhancing tumor accumulation leading to decreased toxicity and improved efficacy [9]. This approach enhances the hydrophobicity of docetaxel through conjugation to highly non-polar fatty alcohols or cholesterol, producing prodrugs composed of the parent drug coupled to the non-polar anchor *via* a hydrolysable linker. Rapid mixing of the hydrophobic prodrugs with appropriate amounts of hydrophobic–hydrophilic block copolymers and a stabilizing phospholipid leads to the spontaneous formation of nanoscale (20–100 nm) solid core nanoparticles [10,11]. Particles generated using this technique have been previously shown to accumulate and remain in tumor tissue

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for extended periods of time due to the enhanced permeability and retention effect (EPR) associated with the use of small particulate delivery vehicles [12]. Thus, the plasma circulation lifetime and tumor bioavailability of docetaxel is dictated by three factors: 1) the distribution properties of the polymer nanoparticle; 2) the hydrolysis kinetics of the ester bond between docetaxel and the linker and 3) the hydrophobicity of the anchor.

As we have previously shown with paclitaxel, the choice of lipid anchor and linker chemistry are the critical factors that determine the plasma elimination rate, rather than the physicochemical properties of the parent drugs themselves [12]. Here we provide an analysis of the hydrolysis rate of docetaxel linked to cholesterol through a diglycolate linkage (Procet 8, Fig. 1) and formulated into phospholipid/copolymer nanoparticles. Although it is desirable to directly measure prodrug hydrolysis rates *in vivo*, it is difficult to quantify this conversion rate as the prodrug undergoes substantial *ex vivo* hydrolysis, and docetaxel has a very high volume of distribution and clearance. Consequently we utilized pharmacokinetic modeling as a tool for defining the *in vivo* hydrolysis rates of Procet 8 in order to better understand the mechanism(s) whereby this formulation provides markedly enhanced efficacy compared to the parent drug.

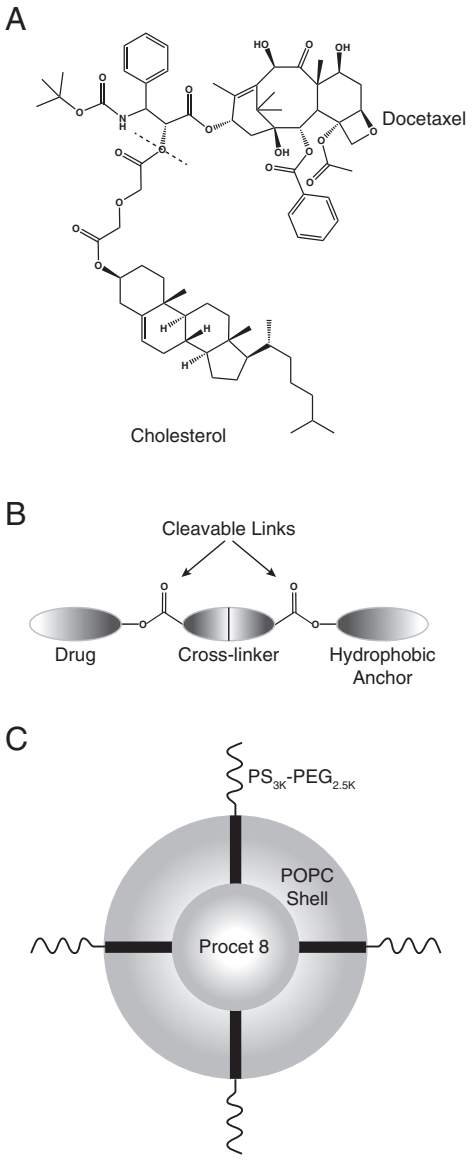

Fig. 1. 3′-Docetaxel diglycolate cholesterol (Procet 8). A.) The Procet 8 molecule is displayed, with the cholesterol and DTX portions of the prodrug labeled. The dashed line shows the site of hydrolytic cleavage to regenerate active docetaxel. B.) A schematic of the Procet 8 molecule, with the hydrophobic anchor, diglycolate linker, and drug portion. C.) A schematic representing the conceptualized micellar Procet 8 nanoparticle is displayed. The inner hydrophobic Procet 8 drug core is surrounded by an outer layer of POPC and $PS_{3K}PEG_{2.5K}$.

Pharmacokinetic modeling and simulation is a powerful tool that has been used previously for prediction of drug concentrations in the absence of analytical techniques that allow for direct quantification [13,14]. A modeling approach was undertaken to predict the DTX plasma profile by fitting plasma profiles of the prodrug and a commercial DTX formulation, Taxotere, to their respective cumulative biliary metabolite data, and thereby describe prodrug metabolism and DTX bioavailability. The modeling of excretion data to predict underlying plasma profiles has been performed successfully before [14]. However, this is the first example that we are aware of in which biliary excretion data has been used for the prediction of drug concentrations for a nanoscale prodrug formulation.

DTX in man and rodents is cleared by CYP3A mediated oxidation followed by biliary excretion, with renal clearance having a minor contribution (<10%) [15–17]. The metabolite docetaxel hydroxy-tert-butyl carbamate (hydroxydocetaxel, HDTX) was chosen for the purpose of modeling plasma concentrations, since analytical standards are available and it is the primary hepatic metabolite in rats and humans [16,17]. Bile duct cannulated Sprague Dawley rats treated with molar equivalent doses of the taxane in the form of Taxotere or Procet 8, had plasma, bile and urine samples analyzed for HDTX, DTX and Procet 8 concentrations by validated LC–MS and –UV methods. The resulting concentration profiles were fit to a two-compartment DTX model, with both linear and non-linear DTX clearance, and linear hydrolysis of the Procet 8 prodrug. This model was then used to simulate the DTX plasma profile, in order to gain insight into the prodrug pharmacokinetic behavior.

2. Materials and methods

2.1. Material

Docetaxel (DTX) was purchased from LC Laboratories. Taxotere (Sanofi-Aventis) was obtained from the NIH pharmacy. DTX hydroxy-tert-butyl carbamate (HDTX) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX). Docetaxel-d9 (DTX-d9) was purchased from Medical Isotopes, Inc. (Pelham, NH). BD Vacutainer PST gel Li-heparin tubes were purchased from Moore Medical (New Britain, CT). Acetonitrile was purchased from VWR (Radnor, PA). Formic acid was purchased from Thermo Scientific (Barington, IL). Alpha-tocopherol was purchased from Sigma-Aldrich (St. Louis, MO). ZORBAX-SB-C18, 5 μ particle, 2.1 × 100 mm and 4.6 × 150 mm column was purchased from Agilent Technologies, Inc. (Santa Rosa, CA), Sunfire C18 5 μM particle, 2.1 × 10 mm and 4.6 × 10 mm guard column was purchased from Waters, Inc. (Milford, MA). Cholesteryl diglycolate was synthesized as previously described [12]. 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Lipoid LLC (Newark, New Jersey). Polystyrene (3000 mwt)-b-polyethylene oxide (2500 mwt) (PS-PEG) was purchased from Polymer Source Inc. (Montreal, Quebec). All other reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario) and used as received. All other solvents were purchased from VWR International (Mississauga, Ontario).

2.2. Synthesis of 3′-docetaxel diglycolate cholesterol (Procet 8, Fig. 1)

A solution of docetaxel (6.12 g), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (1.53 g), 4-N,N-dimethylaminopyridine (1.50 g) and cholesteryl diglycolate (4.46 g) in dichloromethane (80 mL) was stirred at room temperature for 90 min. The reaction was monitored by TLC. The reaction mixture was washed with dilute hydrochloric acid, dried over anhydrous magnesium