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The Effective Solubilization of Hydrophobic Drugs Using Epigallocatechin Gallate or Tannic Acid-Based Formulations

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ABSTRACT

Hydrotropic solubilization of hydrophobic drugs requires supramolar amounts of hydrotropes with potential toxicity issues. We investigated the use of epigallocatechin gallate (EGCG) and tannic acid at millimolar concentrations, as hydrotrope-like solubilizing agents. Paclitaxel, docetaxel, amphotherecin B, curcumin, or rapamycin were dried down with EGCG or tannic acid from ethanol and then redissolved in aqueous media. Following centrifugation and filtration, the drug solubility was measured using HPLC. The uptake of docetaxel into cells from EGCG-based solutions was measured using radiolabeled drugs. Both EGCG and tannic acid effectively increased the aqueous solubility of all drugs from low levels (μg/mL) to high levels (mg/mL) in a concentration-dependent fashion at millimolar concentrations. Solutions were generally stable at room temperature over 24 h. Compared with micellar formulations, EGCG-based solutions of docetaxel demonstrated markedly improved drug uptake or transport levels in all cell lines. The use of these additives may provide improved formulation of various hydrophobic drugs using oral, parenteral, localized, or device-associated delivery systems.

Introduction

There is no universal method for the solubilization of hydrophobic drugs for therapeutic applications. Organic solvents cannot easily be used clinically and cosolvent systems based on agents like propylene glycol generally require unacceptable concentrations of solvents that cannot be used in most practical settings.[1] More sophisticated systems, like those used commercially to deliver the taxane drugs paclitaxel (PTX) (Taxol™) and docetaxel (DTX) (Taxotere™), use combinations of solvents with surfactant molecules to create micellar carriers. Alternatively, polymeric systems, such as diblock copolymer micelles,[2] solid-core nanoparticles,[3,4] hyperbranched polyglycerols,[5] or larger microspheres,[6] have been previously described to deliver PTX or DTX over the last 20 years.

However, drugs work therapeutically as free molecules and need to be presented to target sites as such. Micellar and polymer-based carrier systems provide a barrier to drug access to target sites because, by design, they bind or sequester the drug in the carrier and rely on some sort of release system to liberate the drug as single molecules. This sequestration may reduce the level of drug partitioning into target cells potentially favoring drug clearance over efficacy. Obviously, in some cases, drug retention within the carrier is preferred to provide either controlled release or extended circulation aspects. Therefore, for various clinical applications there exists a preferred balance between optimal solubilization and carrier format.

Hydrotropic solubilization, whereby the inclusion of a second solute increases the aqueous solubility of the hydrophobic compound, is a well-established phenomenon. Because of its clinical relevance, PTX is perhaps the most extensively studied drug in hydrotropic systems. Studies from the laboratory of Kinam Park have described numerous agents (especially nicotinamide derivatives) as effective hydrotropic solubilizers of PTX.[7-9] By definition, hydrotropic agents must be water soluble and only work at high concentrations.[7-11] Although not fully understood, the solubilization mechanism requires the hydrotrope to self-aggregate in solution before drug association and solubilization can occur. This "critical hydrotrope concentration" (also known as the minimum hydrotropic concentration [MHC]) usually occurs at concentrations well above 1 M and is analogous to the critical micelle concentration that occurs with amphipathic molecules such as diblock copolymers or surfactants at micromolar concentrations.[4] This MHC threshold results in nonlinear drug solubilization as a function of hydrotrope

Abbreviations used: DENA, diethyl nicotinamide; DTX, docetaxel; EGCG, epigallocatechin gallate; HBSS, Hanks buffered salt solution pH 7.4; HUVEC, human umbilical vein endothelial cells; MDCK, Madin-Darby canine kidney cells; MePEG-PDLLA, methoxy (polyethylene glycol)-block-poly (D,L, lactic acid); MHC, minimum hydrotropic concentration; PTX, paclitaxel.

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concentration so that the best hydrotropes to date (nicotinamide based) have virtually no solubilization effect for PTX below a concentration of 1 M.[7] Optimally, hydrotropes contain hydrophobic aromatic ring structures (to associate with the drug) balanced with hydrophilic groups to maintain aqueous solubility. It is now clear that hydrophobic interactions between drug and hydrotope may be augmented with hydrogen bonding interactions to improve the solubilization potential.[7,9,10] However, despite the clear ability and advantage of maintaining a drug at the molecular level in solution, workers in the hydrotropy field recognize that any formulation development based on small molecule hydrotropic solubilization is impractical because supramolar concentrations of agents like nicotinamide cannot be administered due to toxicity issues.

In a perfect world, non—carrier-based solubilization methods would involve the use of low concentrations of nontoxic solutes without the need for solute aggregation and would not strongly bind or sequester the drug thus allowing for drug mobility and partitioning into target cells. In this study, we describe the use of epigallocatechin gallate (EGCG) or tannic acid as solute molecules that achieve these goals. Both these agents are listed as food and generally regarded as safe by the US Food and Drug Administration. Not only do these agents provide the concentration-dependent solubilization of PTX and DTX using low millimolar concentrations of solute, they also solubilize numerous other hydrophobic drugs like curcumin, rapamycin, and amphoterecin. The first objective of this study was to characterize the solubilization effect of EGCG and tannic acid with a range of hydrophobic drugs. Second, we compared the uptake of DTX into various cells using either EGCG or tannic acid solutions with micelle carrier-based solubilization systems.

Materials and Methods

Materials

Chemicals and Solvents

HPLC-grade acetonitrile (ACN), dichloromethane, and ethanol were obtained from Fisher Scientific (Fairlawn, NJ). American Chemical Society—grade sodium chloride, magnesium chloride, calcium chloride, sodium dihydrogen orthophosphate, sodium bicarbonate, glacial acetic acid, and anhydrous sodium acetate were purchased from Fisher Scientific (Fairlawn, NJ). Triton X-100 and Cremophor EL were obtained from Fluka BioChemika (Buchs, Switzerland) and Tween 80 (polyoxyethylene-sorbitan monooleate) from Sigma-Aldrich (St. Louis, MO). Liquid scintillation fluid, CytoScint™ ES, was purchased from MP Biomedicals (Irvine, CA). EGCG (>94%) was obtained from DSM (Cambridge, ON). Curcumin, amphoterecin B (both >95%), and tannic acid (American Chemical Society grade) were purchased from Sigma-Aldrich. Transwell cell culture plates (3 μm filters, collagen coated, 12 mm) were obtained from Corning, NY.

PTX and DTX were purchased from Polymed Therapeutics, Inc. (Houston, TX). Tritium-labeled DTX in ethanol was purchased from Moravek Biochemicals (Brea, CA) with a specific activity of 23.2 Ci/mmol. Diblock copolymer, methoxy poly(ethylene glycol)-block-poly(D,L-lactic acid) (MePEG-PDLLA), was provided by Angiotech Pharmaceuticals (Vancouver, BC). The copolymer was manufactured with a weight ratio of 60:40 (MePEG:PDLLA), using a MePEG molecular weight of 2000 g/mol.

Drug Solubilization Experiments

Drugs and EGCG or tannic acid were weighed directly into 15 mL glass tubes and dissolved in 2 mL of ethanol. Excess drug was added to all tubes in this process. The contents were dried down under nitrogen and vacuum dried for 48 h. Samples were then solubilized in either phosphate buffered saline pH 7 (for EGCG) or water (tannic acid) and vortexed at 10-min intervals for 2 h while keeping the samples at either 25°C, 37°C, or 50°C in incubators in the dark. Samples were then centrifuged at 3000× *g* and the clear supernatant was passed through a glass syringe microfilter (1 μm) and stored in a 1 mL capped vial. These centrifugation methods allowed for full sedimentation of insoluble contents and identical results were obtained for samples centrifuged at 14,000× *g*. A 20 μL aliquot was then added to 980 μL of methanol for drug quantitation by HPLC or absorbance spectroscopy analysis. Glass filters were used to avoid any unwanted drug adsorption to plastic filters.

In tannic acid experiments involving PTX, DTX, or amphoterecin, the experiments were repeated in different pH solutions by adjusting the pH of the tannic acid solution with a 2 M sodium hydroxide solution. Curcumin is insoluble in water at low pH but solubility does increase slightly in alkaline conditions. Therefore, the solubilization of this drug was measured at low pH only so that only tannic acid-induced solubilization effects would be measured.

Formulation and Drug Stability Experiments

For 24-h formulation stability experiments, the previously centrifuged and filtered 1 mL samples used in the 2-h experiments were left at 25°C for 24 h in the dark, recentrifuged at 3000× *g* and refiltered. A 20-μL aliquot was then diluted in 980 μL of methanol for drug analysis. To check for possible drug degradation in aqueous media over 24 h, drug solutions were made up at 1 μg/mL (0.5 μg/mL for curcumin) in water and left in sealed tubes for 24 h in the dark. The concentration of the drug in the water was then compared with that in freshly made solutions to investigate drug degradation.

Preparation of Paclitaxel and Docetaxel-Loaded MePEG-PDLLA Micelles

Micelles loaded with DTX were prepared via the solvent evaporation technique. DTX and MePEG-PDLLA copolymer (10% wt/wt) were dissolved in ACN along with a small aliquot of ^{3}H DTX and dried with nitrogen gas. The resulting polymer/drug matrix was reconstituted with Hanks buffered salt solution (HBSS) and adjusted to pH 7.4 (40°C), and vortexed for 2 min and allowed to cool.

Preparation of Docetaxel Formulations for Cell Studies

EGCG or tannic acid solutions were made up by dissolving each agent along with the drug and an appropriate amount of ^{3}H DTX (final concentration 3 μCi/mL) in ACN. Amphoterecin B or curcumin solutions were prepared in the same way without any radioactive drug. All drug and carrier solutions were dried under nitrogen followed by vacuum drying for 2 days. The resulting matrix formed fine films of material on the tube walls. Solutions were made up in HBSS.

Drug Analysis

PTX and DTX were quantitated using liquid scintillation for cell studies and by HPLC for drug solubilization studies. HPLC analysis for PTX, DTX, amphoterecin B, and rapamycin were performed using a Waters HPLC system comprising a NovaPak C18 column, with a mobile phase at a flow rate of 1 mL/min. The mobile phase for PTX and DTX was 58% ACN, 5% methanol, and 37% water with detection at 232 nm. The mobile phase for amphoterecin B was 40% ACN, 4.3% acetic acid, and 55.7% water with detection at 403 nm. The mobile phase for rapamycin was 38% ACN, 34% methanol, and 28% water with detection at 228 nm. Curcumin was quantitated using absorbance analysis at 420 nm.