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Research paper

Lipophilic salts of poorly soluble compounds to enable high-dose lipidic SEDDS formulations in drug discovery

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ABSTRACT

Self-emulsifying drug delivery systems (SEDDS) have been used to solubilize poorly water-soluble drugs to improve exposure in high-dose pharmacokinetic (PK) and toxicokinetic (TK) studies. However, the absorbable dose is often limited by drug solubility in the lipidic SEDDS vehicle. This study focuses on increasing solubility and drug loading of ionizable drugs in SEDDS vehicles using lipophilic counterions to prepare lipophilic salts of drugs. SEDDS formulations of two lipophilic salts—atazanavir-2-naphthalene sulfonic acid (ATV-2-NSA) and atazanavir-dioctyl sulfosuccinic acid (ATV-Doc)—were characterized and their performance compared to atazanavir (ATV) free base formulated as an aqueous crystalline suspension, an organic solution, and a SEDDS suspension, using *in vitro*, *in vivo*, and *in silico* methods. ATV-2-NSA exhibited ~6-fold increased solubility in a SEDDS vehicle, allowing emulsion dosing at 12 mg/mL. In rat PK studies at 60 mg/kg, the ATV-2-NSA SEDDS emulsion had comparable exposure to the free-base solution, but with less variability, and had better exposure at high dose than aqueous suspensions of ATV free base. Trends in dose-dependent exposure for various formulations were consistent with GastroPlus™ modeling. Results suggest use of lipophilic salts is a valuable approach for delivering poorly soluble compounds at high doses in Discovery.

1. Introduction

Driven by the complexity of pharmacological targets, pharmaceutical pipelines are increasingly filled with poorly water-soluble compounds. The factors contributing to this trend include the addition of lipophilic residues to increase ligand-receptor affinity, the broadening of the chemical space using combinatorial chemistry, and the use of high-throughput screening [1,2]. Highly potent compounds may be discovered through these means, but their poor water solubility can lead to numerous challenges during development—particularly, reduced systemic exposure after oral administration [3].

Poorly water-soluble molecules present a particular formulation challenge for high-dose studies—both in Discovery and in later stages of development. In Discovery, high-dose studies are invaluable in aiding early identification of toxicity—the leading cause of attrition at all stages of drug development [4]. High doses are also required in pharmacokinetic (PK) studies, especially toxicokinetic (TK) studies, where high-exposure multiples are required to evaluate safety margins over the entire range of efficacious exposure.

Advances in the pharmaceutical sciences have produced numerous approaches to address the challenges presented by poorly water-soluble compounds, which are designated Class II and IV compounds in the Biopharmaceutics Classification System (BCS). These strategies include reducing particle size to increase

Abbreviations: 2-NSA, 2-naphthalene sulfonic acid; ATV, atazanavir; ATF-2-NSA, atazanavir-2-naphthalene sulfonic acid; ATV-Doc, atazanavir-dioctyl sulfosuccinic acid; Doc, dioctyl sulfosuccinic acid.

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the surface area and dissolution rate [5]; solubilization in cosolvents [6]; complexation [7,8]; formulation as solid amorphous dispersions [9]; formation of salt forms [10]; and the use of lipidic systems for lipophilic drugs [11]. Each approach has its limitations. For example, reducing particle size has little impact on solubility-limited exposure, solubilization in cosolvents is restricted to a limited range of pharmaceutically acceptable excipients, and the binding constant of inclusion complexes is often insufficient to solubilize the required dose using acceptable amounts of complexing agents. Solid amorphous dispersions offer improved solubilization, but are not as facile to prepare as liquid lipid-based formulations.

The formation of salt forms and the use of lipidic systems for lipophilic drugs offers particular promise in addressing the need for stable, high-dose formulations. Salt formation using small, often inorganic, counterions is a well-established technique to increase the dissolution rate and kinetic solubility of drug compounds [12–17]. Although these high-energy salts can increase the oral bioavailability of poorly soluble ionizable compounds, this approach can be limited by the disproportionation of salt to the free-drug form, with concomitant crystallization to a low-energy crystal. To prevent this, many investigators have proposed modifying the physiochemical properties of poorly water-soluble drug compounds using lipophilic counterions to form lipophilic salts (sometimes referred to as "ionic liquids") as an alternative to high-energy salt forms, to enable more effective formulation, delivery, and oral absorption [18–23].

Lipid-based formulations have numerous desirable features; they are easy to manufacture and can increase stability, enhance absorption, and improve bioavailability [24]. However, the drug solubility in the lipid often limits the maximum dose deliverable using lipid vehicles. It would be advantageous to extend the applicability of lipid formulations to a broader range of compounds, particularly those with lower log P values (e.g., between 2 and 5) and higher melting-point (T_m) values, such as atazanavir, the model drug used in the work described here.

Lipophilic ion-drug pairs can markedly increase solubility in lipidic phases through a combination of two mechanisms: (1) the lipophilicity of the counterion, which can help pull the drug into a lipid phase; and (2) the reduced crystalline forces (and T_m) of the ion-drug pair, which tends to increase solubility in all solvents. The increased lipid solubility of such salts can facilitate development of stable, high-concentration formulations for high-dose pharmacokinetic or toxicokinetic studies, which in turn can result in higher exposures than those achievable with formulations of other salts or free forms of the drug. Reports have detailed the use of lipophilic counterions to form ion-drug pairs that enable higher drug loadings in solid lipid nanoparticles [25–27] or into oil-in-water emulsion droplets [28]. More recently, Shadid et al. showed a 2.5-fold increase in oral exposure in rats for sulfasalazine when formulated as an "ionic liquid" [23]. In addition, Sahbaz et al. demonstrated improved oral exposure in rats for three poorly soluble compounds by formulating them with a variety of lipophilic counterions [22].

The purpose of the work described here is to demonstrate the feasibility of using lipophilic ion-drug pairs (i.e., salts) in lipid vehicles to enable dosing of poorly water-soluble drugs at high concentrations for preclinical studies. Demonstrating that this promising approach is suitable for high-dose testing, particularly in a Discovery setting, would be valuable, since solubilizing high doses of such drugs is difficult in lipid vehicles using free-acid/free-base drug forms or typical high-energy salt forms.

This study describes the use of lipophilic counterions to prepare lipophilic salt forms of a weakly basic drug—atazanavir (ATV)—to increase drug solubility in a self-emulsifying drug-delivery system (SEDDS) vehicle (above that achievable with the free base and typical inorganic salts of the drug) to increase the deliverable dose, enabling high-dose testing. The synthesis, characterization, formulation development, and *in vivo* PK profiles of the lipophilic salts atazanavir 2-naphthalene sulfonic acid (ATV-2-NSA) and atazanavir-dioctyl sulfosuccinic ("docusate") acid (ATV-Doc) are described. ATV, which is an antiretroviral drug of the protease inhibitor class [29–32], was chosen as a model compound due to its low aqueous solubility (~1.5 μg/mL at pH 6.5) and weakly basic pKa (~4.6). 2-naphthalene sulfonic acid (2-NSA) and dioctyl sulfosuccinic acid (Doc) were chosen as counteracids because of their low pKa values [16], which result in complete protonation of ATV at intestinal pH, as well as for their pharmaceutical precedence [33,34] and low toxicity [35]. The SEDDS formulation chosen is typical of a Type II formulation in the lipid formulation classification system (LFCS) described in the literature [36,37], but was not optimized for ATV.

2. Materials and methods

2.1. Materials

All reagents and solvents were used as received. ATV was obtained from Bristol-Myers Squibb (New York, New York). 2-NSA was purchased from TCI Fine Chemicals (Portland, Oregon). Docusate sodium and polysorbate 80 (Tween® 80) were purchased from Spectrum Chemical (Gardena, California). Tetrahydrofuran (THF, anhydrous) and hydrochloric acid (HCl, 1.25 M) in methanol were obtained from Aldrich Chemical (Milwaukee, Wisconsin). Miglyol® 812, a mixture of medium-chain triglycerides, was a kind gift from Cremer Oleo (Hamburg, Germany). Glyceryl monocaprate (Capmul® MCM C10) was obtained from Abitec Corporation (Columbus, Ohio). Dimethylacetamide (DMAC), polyethylene glycol 400 (PEG 400), and d_6 dimethyl sulfoxide (d_6 DMSO, 99.8% deuterated) were purchased from Sigma Aldrich (Belgium and USA). Hydroxypropyl β cyclodextrin (Kleptose® HPB) was sourced from Roquette (France). Citric acid monohydrate and sodium hydroxide (NaOH) were purchased from Merck (India). High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) and methanol were obtained from Honeywell, Burdick, and Jackson (Muskegon, Michigan). Trifluoroacetic acid (TFA) was purchased from Thermo Scientific (Rockford, Illinois). Phosphate butter solution (PBS) consisted of 82.1 mM NaCl, 20 mM Na_2HPO_4-$\cdot 7H_2O$, and 46.7 mM KH_2PO_4, adjusted to pH 6.5 with 30% NaOH, and had an osmolarity adjusted to 290 mOsm/kg with 1:20.4 KCl:NaCl. Methylcellulose (Methocel™ A4M) was a kind gift from The Dow Chemical Company.

2.2. Methods

2.2.1. Preparation of amorphous ATV free base

Amorphous ATV free base was prepared by spray-drying ATV free base from solution on a custom mini spray dryer designed and built at Bend Research, a division of Capsugel [9]. ATV free base was dissolved in methanol at a concentration of 18 mg/mL and spray-dried at an inlet temperature of 65 °C and a nitrogen flow rate of 30 standard liters per min.

2.2.2. Preparation of ATV salts

The ATV salts were prepared quantitatively by stoichiometric addition of strong acids to the ATV free base. The 2-NSA and Doc salts were both isolated in the amorphous form. The 2-NSA salt was also separately isolated as crystalline material. The Doc salt was not easily isolated in the crystalline form, so only the amorphous form was characterized.