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Research paper

Predicting biopharmaceutical performance of oral drug candidates – Extending the volume to dissolve applied dose concept

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ABSTRACT

The purpose of the study was to experimentally deduce pH-dependent critical volumes to dissolve applied dose (VDAD) that determine whether a drug candidate can be developed as immediate release (IR) tablet containing crystalline API, or if solubilization technology is needed to allow for sufficient oral bioavailability. pH-dependent VDADs of 22 and 83 compounds were plotted vs. the relative oral bioavailability (AUC solid vs. AUC solution formulation, Frel) in humans and rats, respectively. Furthermore, in order to investigate to what extent Frel rat may predict issues with solubility limited absorption in human, Frel rat was plotted vs. Frel human. Additionally, the impact of bile salts and lecithin on in vitro dissolution of poorly soluble compounds was tested and data compared to Frel rat and human. Respective in vitro – in vivo and in vivo – in vivo correlations were generated and used to build developability criteria. As a result, based on pH-dependent VDAD, Frel rat and in vitro dissolution in simulated intestinal fluid the IR formulation strategy within Pharmaceutical Research and Development organizations can be already set at late stage of drug discovery.

1. Introduction

In order to bring innovative drug products to the patient in need quickly and in a cost-effective manner, it is a key within Research & Development (R & D) organizations to focus on the right formulation strategy early-on [6,19]. The type of formulation needed, e.g. immediate release (with or without solubilization technology), enteric coating, slow release etc., depends on dose, pharmacokinetic profile as well as the physicochemical properties of the API. The most frequent challenge, though, is the ever increasing number of poorly soluble drug candidates [11,20,31]. In addition, the terms "poor solubility" or "unfavorable dose/solubility ratio" have not been clearly defined in a biopharmaceutical performance context. Some of the definitions were derived hypothesis-based on GI tract fluid volumes/water intake with tablet administration and transit times: "poorly soluble when dose does not dissolve in 250 ml of pH 1–7" [1,30,26]. Other definitions were derived based on in vitro/in vivo observations from a limited number of compounds and formulations: "daily dose does not dissolve in 500 ml of aq. media (pH 1–7) for poorly permeable compounds and >10 L for highly permeable compounds" [4]. Furthermore, some definitions were mentioned without further reasoning: "<100 mg/L often present dissolution limitation to absorption" [14], "solubility of 10–100 mg/L received a medium risk on Aventis score card" [2], "if dose/solubility ratio is >1000 ml, even in the presence of favorable physiological factors (pH, bile salts), the solubility is likely to cause problems with bioavailability" [8], and "practically insoluble: <100 mg/L", [10]. The aforementioned low solubility/unfavorable dose/solubility descriptions do not take into account the potentially positive impact of pH-dependent solubility of poorly soluble bases and acids on in vivo dissolution, and thus, on oral absorption. More recently however, pH-dependency was explored to a greater extent. For example, based on theoretical modeling work Tsume et al. proposed pH-dependent subclasses within the BCS [29], while Muenster et al. demonstrated experimentally that pH-dependent solubility and dissolution can contribute to complete in vivo dissolution even of drugs that were poorly soluble according to the BCS classification [22].

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This being said, a straight forward correlation of experimentally derived in vivo dissolution data in humans or animals of larger compounds sets vs. in vitro dose/pH dependent solubility or dissolution data is still lacking in the literature. Such in vitro-in vivo correlations (IVIVCs) would allow one to establish certain pH-dependent volumes to dissolve applied dose (VDAD), which could guide the pharmaceutical scientists in their choice of formulation. E.g., a standard question at late research/early development stage within pharmaceutical R & D organizations is: "Can we go with a standard immediate release tablet containing the crystalline API, or do we need solubilization technology?"

To address this question, the study presented here will show pH-dependent VDADs of 22 crystalline compounds correlated to oral tablet vs. solution pharmacokinetic data in humans (Frel human). Furthermore, in search for a predictive in vivo animal model, Frel rat (oral suspension vs. solution) was plotted vs. Frel human. Respective IVIVCs were generated and used for creating a developability decision tree.

2. Materials and methods

2.1. Test compounds, chemicals and materials

84 compounds (named 1–84) of past and current Bayer research programs were included in the study. Table 1 reveals their molecular weights, *c* log P values (Certara, formerly Tripos [28]), topological polar surface area (TPSA, [9], pK_a for strongest acid and base calculated in ADMET Predictor v7 from Simulation Plus [12], and mean similarities of all molecules to each single molecule expressed by Tanimoto coefficients calculated from MDL MACCS public keys ([5], Table 1). Caco-2 cells were purchased from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (ACC 169, DSMZ; Braunschweig, Germany). 24-well microporous polycarbonate insert filter plates (0.4-μm pore size, Corning Costar, Inc., Cambridge, MA, USA), cell culture media, fetal bovine serum, and antibiotics were purchased from Invitrogen (GIBCO/Invitrogen, Karlsruhe, Germany). FaSSIF (Fasted State Simulated Intestinal Fluid) powder was purchased from Biorelevant (Croydon, UK). All other chemicals were purchased from Sigma Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

2.2. Generation of crystalline test compounds

Preliminary solubility experiments in acetonitrile, dichloromethane, tetrahydrofurane, toluene, ethylacetate, acetone, 2-propanol, 1,4 dioxane, tert-methyl-butyl-ether, diisopropylether, cyclohexane, *n*-pentane, and methanol were conducted in order to identify appropriate crystallization solvents for each compound. A solvent was considered appropriate when approximately 10% of the compound was dissolved in a given volume. Compounds were then stirred for seven days in their respective solvents chosen for crystallization. After stirring, compounds were dried at room temperature (RT) for 72 h and analyzed for solvate formation using head-space gas chromatography (HS 40XL HS-sampler in combination with HP 5890 GC, PerkinElmer/Agilent, Rodgau-Jügesheim/Böblingen, Germany). If solvate formation occurred, stirring was repeated in a different solvent until compounds were solvate free. Then, compounds were subjected to solid state analytics. Crystallinity of the compounds was confirmed by X-ray powder diffraction (XRPD, stoe transmission diffractometer, Stoe&Cie, Darmstadt, Germany) and differential scanning calorimetry (DSC, Diamond-DSC, Perkin Elmer, Rodgau-Jügesheim, Germany). Respective crystalline solid state phases used for solubility and apparent dissolution rate determination were the same as were used for in vivo studies in humans and rat.

2.3. Thermodynamic solubility

Thermodynamic solubility was determined using the shake-flask method based on OECD Guideline 107 [23]. Crystalline, solvate free compounds were added in excess to aqueous media (phosphate buffer, pH 7 (United States Pharmacopoeia, USP), acetate buffer, pH 4.5 (USP), 0.1 M HCl pH 1) and stirred for 16 h at 25 °C. Suspensions were centrifuged and filtered (regenerated cellulose, BRAUNRAND H, RC, 0.45 μm, SPARTAN 13/0,45 RC, D = 13 mm, GE Healthcare Whatman, Germany), and respective filtrates as well as 1:10 and 1:100 dilutions of the filtrates were analyzed by high-performance liquid chromatography (HPLC, Agilent 1100, Agilent, Waldbronn, Germany). Appropriate analytical HPLC methods were developed upfront.

2.4. Compound micronization and particle size measurement

Compounds were micronized using an air jet mill (LSM50, high-quality steel; injector nozzle, 1.1 mm; diffuser, 3.8–5.7 mm; milling nozzle, 0.7 mm; small outlet, 9.7 mm; large outlet, 13.0 mm; injector air pressure, 4.5 bar; milling air pressure, 4.0 bar; throughput, 1 g/min; Bayer Technology, Leverkusen, Germany). In order to assess whether the micronization process had any influence on the crystallinity of the compounds, Raman spectra (RFS 100/S, Bruker, Ettlingen, Germany) and DSC curves (DSC, Diamond-DSC, Perkin Elmer, Rodgau-Jügesheim, Germany) were generated before and after micronization. Particle size of micronized compounds was determined using a Sympatec Rodos SR (Sympatec, Clausthal-Zellerfeld, Germany).

2.5. Apparent dissolution rates (ADRs)

ADRs were determined using the mini-flow-through-cell (USP apparatus IV, Sotax, Basel, Switzerland), equipped with 400 μl cells. Before filling the cells with glass beads and API, a Whatman® glass microfiber filter membrane (Whatman, Kent, United Kingdom) was placed on the bottom of the cell. Then, 30% of the cell volume was filled with 0.1 mm glass beads (Fisher Scientific, Germany). On top of that was placed 1 mg of API. The API was then covered by a thin layer of 0.1 mm glass beads, followed by filling of another 30% of the cell volume with 1 mm glass beads (Sartorius, Germany). Then, the mixture of API, 0.1 mm glass beads, and 1 mm glass beads was mixed with a spatula until homogeneity was achieved, according to visual inspection. Then the remaining free cell space was filled with 0.1 mm glass beads, and the cell covered on top with the same Whatman® glass microfiber filter membrane as was used to cover the bottom of the cell. Flow-through-cells were then equilibrated to 37 °C in a water bath. Finally, a flow rate of 2 ml/min using aqueous media (phosphate citrate buffer, pH 6.8, 50 mOsm/kg; acetate buffer, pH 4.5, osmolarity, 75 mOsm/kg; 0.1 M HCl, pH 1, and FaSSIF) was applied to the flow-through-cells, and 2 min fractions of 4 ml each were collected up to 14 min after the start of the experiment. The amount of dissolved compound in each fraction was determined by HPLC (HP1100, Waldbronn, Böblingen, Germany), and apparent dissolution curves were generated.

2.6. Permeability across Caco-2 cells

Permeability across Caco-2 cell monolayers was determined as described elsewhere [33]. Briefly, Caco-2 cells were seeded at a density of 40,000 cells/well and grown for 15 days on 24-well filter insert plates with medium change every 4 days. Compounds were added at a final concentration of 2 μM to either the apical (A) or basolateral (B) compartment of insert filter plates. Compound concentrations in both the apical and basolateral (B) compartments at