Forecasting the In Vivo Performance of Four Low Solubility Drugs from Their In Vitro Dissolution Data

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Purpose. To assess the usefulness of biorelevant dissolution tests in predicting food and formulation effects on the absorption of four poorly soluble, lipophilic drugs.

Methods. Dissolution was studied with USP Apparatus II in water, milk, SIFp, FaSSIF, and FeSSIF. The in vitro dissolution data were compared on a rank order basis with existing in vivo data for the tested products under fasted and fed state conditions.

Results. All drugs/formulations showed more complete dissolution in bile salt/lecithin containing media and in milk than in water and SIFp (USP 23). Comparisons of the in vitro dissolution data in biorelevant media with in vivo data showed that in all cases it was possible to forecast food effects and differences in absorption between products of the same drug with the physiologically relevant media (FaSSIF, FeSSIF and milk). Differences between products (both in vitro or in vivo) were less pronounced than differences due to media composition (in vitro) or dosing conditions (in vivo).

Conclusions. Although biorelevant dissolution tests still have issues which will require further refinement, they offer a promising in vitro tool for forecasting the in vivo performance of poorly soluble drugs.

KEY WORDS: dissolution; low solubility drugs; troglitazone; atovaquone; sanfretinem cilexetil.

INTRODUCTION

The usefulness of in vitro dissolution data in predicting the in vivo performance of drugs with dissolution limited absorption remains an open issue (e.g., 1). Dissolution is a dynamic process which is strongly dependent on both the composition of the medium and the hydrodynamics. Since the luminal environment in the proximal gastrointestinal (GI) tract varies considerably with site and meal ingestion, it is worth considering the use of several different sets of dissolution conditions to arrive at a complete picture of how an immediate release (IR) dosage form will release its active component under various dosing conditions. We have recently shown that with the amount of information available today on GI physiology and the composition of the GI contents, it should be possible to design a suitable set of tests to predict the in vivo dissolution of both class I and class II drugs from IR drug products (2,3).

In the present study we assessed the usefulness of biorelevant in vitro dissolution data in forecasting the in vivo performance of four lipophilic, sparingly soluble drugs on an a priori basis. That is, the in vitro tests were carried out and the in vivo absorption behavior predicted before the bioavailability data were made available from the manufacturer.

The physicochemical characteristics of the drugs studied and the drug content of the relevant immediate release tablets tested in the present study are shown in Table 1. Troglitazone is an antidiabetic used in non-insulin diabetes mellitus (4), atovaquone is an antiprotozoal agent (5) and sanfretinem cilexetil is a prodru of sanfretinem, a molecule with antibiotic properties (6,7). GV150013X is a benzodiazepine with a molecular formula of C23H27N2O2.HCL (GlaxoWellcome data on file). The four drugs tested in this study were chosen for several reasons. First, we wanted to determine how useful the media are for drugs that are representative of the challenges currently being faced by Pharmaceutical R&D groups, rather than using classical examples of poorly soluble drugs. Second, in vitro results are of little use in evaluating new media without the corresponding in vivo data. For all four drugs, appropriate bioavailability data with formulation comparisons and/or food effects were on file at the manufacturer. And third, the four compounds chosen were all purported to fall under class II of the Biopharmaceutics Classification Scheme, the class for which in vitro/in vivo correlations are most likely to be obtainable (8).

MATERIALS AND METHODS

Materials

Troglitazone, atovaquone, trans-2-hydroxy-3-(4-phenylethoxy)-1,4-naphthoquinone (internal standard for the atovaquone assay) and GV150013X, all in powder form, were supplied by Glaxo Wellcome R&D, UK. Powder drug substance of sanfretinem cilexetil was supplied by Glaxo Wellcome S.p.A., Verona, Italy. Samples from three different IR tablet formulations of troglitazone [formulation M94/055C (Romozin®), formulation D157/155B, and formulation D157/155D], one IR tablet formulation of atovaquone (Wellvone®, lot # C3377A) and one IR tablet formulation of GV150013X (lot # M95/105A) were provided by Glaxo Wellcome R&D, UK. Samples from two different IR tablet formulations of sanfretinem cilexetil (codes 630/C078/49 and 630/C091/59) were provided by Glaxo Wellcome S.p.A., Verona, Italy.

9-Acetylanthracene (internal standard for the troglitazone assay), and sodium taurocholate 98% pure lot # 15H5001 were purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Egg phosphatidylcholine, Lipoid E PC 99.1% pure, lot # 12091-1, was a gift from Lipoid GmbH (Ludwigshafen, Germany). Potassium dihydrogen phosphate, and potassium chloride, all Analytical Grade, were purchased from E. Merck (Darmstadt, Germany). The source of the long life milk, 3.5% fat, was Landesgenossenschaft Ennstal Molkerei-Betriebe (Steinach, Austria).
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Table 1. Physicochemical Characteristics of the Four Drugs and Drug Content of Their Immediate Release Tablets Tested in this Study (GlaxoWellcome Data on File)

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Nature</th>
<th>Molecular weight</th>
<th>pKa(s)</th>
<th>Aqueous solubility (µg/ml)</th>
<th>LogP*</th>
<th>Single dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troglitazone</td>
<td>Weak acid</td>
<td>441.5</td>
<td>6.1 and 12.0</td>
<td>1.93a</td>
<td>2.7</td>
<td>200</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>Neutral†</td>
<td>366.8</td>
<td>Not applicable†</td>
<td>0.430a†</td>
<td>5.1</td>
<td>250</td>
</tr>
<tr>
<td>Sanfotericin cilexetil</td>
<td>Neutral</td>
<td>368.4</td>
<td>Not applicable</td>
<td>180†</td>
<td>3.0 and 3.1†</td>
<td>400</td>
</tr>
<tr>
<td>GV150013X</td>
<td>Neutral†</td>
<td>534.7</td>
<td>Not applicable†</td>
<td>0.160†</td>
<td>5.4</td>
<td>1</td>
</tr>
</tbody>
</table>

* In octanol-water.
† pH 7, ambient temperature, crystalline powder.
‡ Under physiological conditions.
§ From dissolution results of the tested product (which contains the drug in crystalline form) in water.
¶ pH 7, 37°C, crystalline powder.
‖ Two diastereoisomers.
** SIF<sub>7q</sub> 37°C, crystalline powder.

Methods

Dissolution tests were performed with the USP 23 Apparatus II using a Pharma Test dissolution tester (Type PTW SHI-PTW S3C) and employing 500 ml of dissolution medium at a temperature of 37 ± 0.5°C. Experiments were run in triplicate at 100 rpm. Dissolution tests were performed at two sites, the University of Athens and the University of Frankfurt. Inter laboratory reproducibility was confirmed for every product by running dissolution tests in USP 23 Simulated Intestinal Fluid without pancreatin (SIF<sub>7q</sub>), at both sites.

Three to five ml samples were withdrawn at appropriate times, using a 5 ml Fortuna Optima® syringe (Fischer Labor technik, Frankfurt/Main, Germany) fitted with stainless steel tubing to facilitate representative sampling with sample replacement. Aqueous samples were filtered through 0.45 µm filters, chosen in each case for their lack of adsorption of the compound in question. In cases where adsorption of to filters was inevitable (for the highly lipophilic compounds atovaquone and GV150013X (Table 1)) appropriate corrections were made. Milk’s composition does not allow the use of filters with small pore size, so a compromise between pore size and filtering efficiency had to be found. Other investigators have previously used dialysis techniques for studying the dissolution of IR products and solubility or drug powders in 75% milk using membranes with 20 µm pore sizes (9,10). Coefficients of variation from such studies were always less than 10% indicating minimal possibility of inadequate separation of the solid particles. In the present study, milk samples were filtered through double Whatman® filters with a pore size of 8 µm (No. 40). Adsorption onto these filters was substantial only in the case of atovaquone samples, for which appropriate corrections were made.

Composition of Various Dissolution Media

Dissolution experiments with all products were run in water, long life whole milk (3.5% fat), USP 23 simulated intestinal fluid without pancreatin (SIF<sub>7q</sub>), fasted state simulating intestinal fluid (FaSSIF), and fed state simulating intestinal fluid (FeSSIF). Composition of all dissolution media was identical with that described previously (3).

Analytical Methods

All assays were performed by HPLC using a UV detector.

Troglitazone Assay. In all cases the mobile phase comprised of 60:40:0.08 acetonitrile:water:orthophosphoric acid, the flow rate was 1.4 ml/min, and troglitazone was detected at 230 nm. Solutions were protected from light (11). For experiments in water and SIF<sub>7q</sub>(Athens), 50 µl of appropriately diluted samples containing 9-Acetylanthracene (internal standard) were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column. For experiments in SIF<sub>7q</sub>(Frankfurt), FaSSIF and FeSSIF, 20 µl of appropriately diluted samples were injected onto an Alltech 250 × 4.6 mm Lichrosorb ODS-5 column. For experiments in milk, each sample was treated with acetonitrile and sodium hydroxide 2N, the internal standard was added, and the resulting solution was extracted with ethylacetate/hexane 9:1. The organic layer was evaporated to dryness, reconstituted with methanol, and 50 µl were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column.

Atovaquone Assay. In all cases the mobile phase comprised of 30:70 0.4% trifluoroacetic acid in water:acetonitrile, and atovaquone was detected at 254 nm. For experiments in water and SIF<sub>7q</sub>(Athens), 50 µl of appropriately diluted samples containing trans-2-hydroxy-3-(4-phenylcyclo-hexyl)-1,4-naphthoquinone (internal standard) were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column and the flow rate of the mobile phase was 1.5 ml/min. For experiments in SIF<sub>7q</sub>(Frankfurt), FaSSIF, and FeSSIF, 100 µl of appropriately diluted samples were injected onto a Merck Hibar® 125 × 4.0 mm Lichrosorb® RP-8 (5 µm) column and the flow rate of the mobile phase was 0.6 ml/min. For experiment in milk, each sample was treated with 0.5 M acetic acid, the internal standard was added, and the resulting solution was extracted with 2% isooamylic alcohol in hexane. The organic layer was evaporated to dryness, reconstituted with methanol, and 50 µl were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column. The flow rate of the mobile phase was of 1.5 ml/min.

Sanfotericin Cilexetil Assay. In all cases the mobile phase comprised of 55:45:0.28 acetonitrile:100 mM ammonium acetate buffer pH 5.0:glacial acetic acid, and sanfotericin cilexetil...