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

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Received August 27, 1999; accepted September 14, 1999

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, SIF_{sp}, 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 SIF_{sp} (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; sanfetrinem 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 sanfetrinem cilexetil is a prodrug of sanfetrinem, a molecule with antibiotic properties (6,7). GV150013X is a benzodiazepine with a molecular formula of $C_{23}H_{27}N_3O_3.2HCL$ (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-phenyl-cyclo-hexyl)-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 sanfetrinem cilexetil was supplied by Glaxo Wellcome S.p.A., Verona, Italy. Samples from three different IR tablet formulations of troglitazone [formulation M94/058C (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 sanfetrinem 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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0724-8741/99/1200-1876$16.00/0

Table 1. Physicochemical Characteristics of the Four Drugs and Drug Content of Their Immediate Release Tablets Tested in this Study (GlaxoWellcome Data on File)

Drug name	Nature	Molecular weight	Pka(s)	Aqueous solubility (μg/ml)	LogP[a]	Single dose (mg)
Troglitazone	Weak acid	441.5	6.1 and 12.0	1.93[b]	2.7	200
Atovaquone	Neutral[c]	366.8	Not applicable[c]	0.430[d]	5.1	250
Sanfetrinem cilexetil	Neutral	368.4	Not applicable	180[e]	3.0 and 3.1[f]	400
GV150013X	Neutral	534.7	Not applicable	0.160[g]	5.4	1

[a] In octanol-water.
[b] pH 7, ambient temperature, crystalline powder.
[c] Under physiological conditions.
[d] From dissolution results of the tested product (which contains the drug in crystalline form) in water.
[e] pH 7, 37°C, crystalline powder.
[f] Two diastereoisomers.
[g] SIF_{sp}, 37°C, crystalline powder.

Methods

Dissolution tests were performed with the USP 23 Apparatus II using a Pharma Test dissolution tester (Type PTW SIII-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_{sp}), at both sites.

Three to five ml samples were withdrawn at appropriate times, using a 5 ml Fortuna Optima® syringe (Fischer Labortechnik, 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 on 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 0.75% milk using membranes with 20 μm pore sizes (9,10). Coefficients of variation from such studies were always less 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_{sp}), 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_{sp}(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_{sp}(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_{sp}(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_{sp}(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% isoamylic 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.

Sanfetrinem 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 sanfetrinem cilexetil

was detected at 279 nm. For experiments in water and SIF_{sp}(Athens), 50 μl of appropriately diluted samples were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column, and the flow rate of the mobile phase was 1.3 ml/min. For experiments in SIF_{sp}(Frankfurt), FaSSIF, and FeSSIF, 50 μl of appropriately diluted samples were injected onto a Merck Hibar® 125 × 4.0 mm Lichrospher ODS-5 column, and the flow rate was 1 ml/min. For experiments in milk, each sample was treated with acetonitrile, and 50 μl were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column. The flow rate of the mobile phase was 1.3 ml/min.

GV150013X Assay. In all cases GV150013X was detected at 235 nm. For experiments in water and SIF_{sp}(Athens) 100 μl of appropriately diluted samples were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column. The mobile phase was 60:5:12:23 methanol:tetrahydrofurane:acetonitrile:ammonium dihydrogen phosphate 0.025 M buffer (pH 3) and the flow rate was 1.3 ml/min. For experiments in SIF_{sp}(Frankfurt), FaSSIF, and FeSSIF, 100 or 200 μl of appropriately diluted samples were injected onto a Merck Lichrocart® 125 × 4.0 mm Lichrospher® 60RP-select B (5 μm) column, and eluted with a mobile phase comprised of 60:40 acetonitrile:ammonium acetate 67.5 mM buffer (pH 7.5) at a flow rate of 1 ml/min. For experiments in milk, each sample was treated with acetonitrile and 100 μl were injected onto a Spherisorb S5-ODS2 (250 × 4.6 mm) column. The mobile phase comprised of 60:5:12:23 methanol:tetrahydrofurane:acetonitrile:ammonium dihydrogen phosphate 0.025 M buffer (pH 3) and the flow rate was 1.3 ml/min.

For all drugs, linear standard curves were constructed for every sample set. Coefficients of determination were at least 0.990, none of the intercepts were statistically significant, and the coefficient of variation of the slopes in a specific medium was a maximum of 12.0% for troglitazone, 14.3% for atovaquone, 4.4% for sanfetrinem cilexetil and 4.2% for GV150013X.

RESULTS

Troglitazone

Figure 1 shows the mean dissolution profiles of troglitazone from the three different IR products studied in various media. Apart from the milk data of the D157/155D tablets which had a coefficient of variation (CV) of up to 40%, the CV was always less than 20% with the highest values observed at the early sampling times. Dissolution of troglitazone in simple aqueous media is poor, due to the insufficiently basic pH (water and SIF_{sp}) and insufficient buffer capacity (water). Percent dissolved vs. time plots for formulations D157/155B and D157/155D showed a peak in both water and SIF_{sp}. In all three products, the drug is in a solid dispersion form and predominantly amorphous in nature but they differ in the excipients used (GlaxoWellcome data on file). Recrystallization during dissolution testing has been shown to occur and increases with pH, although the rate is retarded by nucleation inhibitors (12) which are included in the formulations (GlaxoWellcome data on file). Therefore, the peak in the dissolution curve is probably due to the supersaturation of the medium with respect to the crystalline drug and its subsequent precipitation. For the other media, solubilization effects and a possible retardation of crystal growth by their surfactant components (12) appear to have prevented the occurrence of a peak phenomenon.

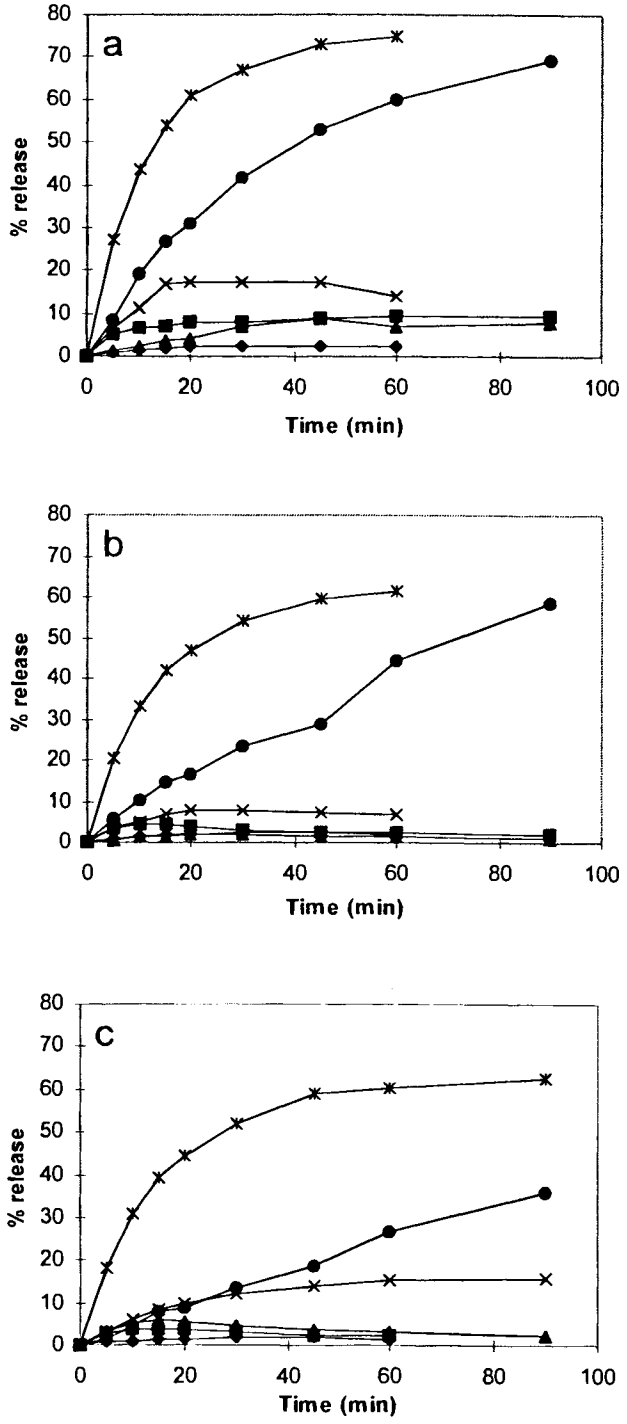

Fig. 1. Mean dissolution profiles of troglitazone in various media from three different IR tablets. (a) Romozin® tablets (lot# M94/058C); (b) formulation D157/155B; (c) formulation D157/155D. -◆- water; -■- SIF_{sp}(Athens); -▲- SIF_{sp}(Frankfurt); -✕- FaSSIF; -✳- FeSSIF; -●- milk.

Results in FaSSIF, FeSSIF, and milk suggest that bile salt and lecithin concentrations as well as milk components can have a large effect on the dissolution characteristics of the compound from the three products. The lower pH of FaSSIF,

compared to SIF_{sp}, is more than compensated for by solubilization effects contributed by bile salt and lecithin. Changing the medium to FeSSIF results in yet better dissolution despite the even lower pH. The maximum % dissolved values in FaSSIF and FeSSIF were 17 and 75 (Romozin®), 7.9 and 61 (D157/155B), and 16 and 63 (D157/155D), respectively. Compared to the profiles in FaSSIF or FeSSIF (which plateaued within 60 min), the profiles in milk exhibit apparent zero order kinetics for the D157/155B, and D157/155D products, indicating that sink conditions are maintained during the study period.

The data obtained in physiologically relevant media predicted that Romozin® might tend to exhibit better absorption than the other two products, and, regardless of the formulation, troglitazone's bioavailability should be higher when administered in the fed state (Fig. 1). These *a priori* predictions concurred with the results of various *in vivo* studies performed in healthy subjects. Data from various bioequivalence studies in the fed state show that their relative bioavailabilities versus the marketed product in the U.S. (i.e., vs. Rezulin®) are 98% for Romozin®, 87% for the D157/155B product, and 58% for the D157/155D product (GlaxoWellcome data on file). Furthermore, Noscal® tablets [which contain 400 mg troglitazone in a solid dispersion form, predominantly amorphous in nature, i.e., in the same form as in the products studied, and which are bioequivalent to Romozin® tablets (GlaxoWellcome data on file)], show a pronounced food effect. The geometric mean values of area under the plasma concentration-time curve (to the last measurable time point, AUC_{last}), and the peak plasma concentration (C_{max}) were both significantly higher, by 59% and 72%, respectively, when the tablet was given 30 min. after food rather than in the fasted state (13).

Atovaquone

Figure 2 shows the mean dissolution profiles of Wellvone® tablets in various media. With the % dissolved being extremely low the variability of the data in simple aqueous media (SIF_{sp}, water) reached as much as 100%. In FaSSIF and FeSSIF, CV values were as high as 36% in the first sampling time point but they stayed lower than 10% at later time points. In milk, CV values ranged from 11–45%. As shown in Fig. 2, dissolution

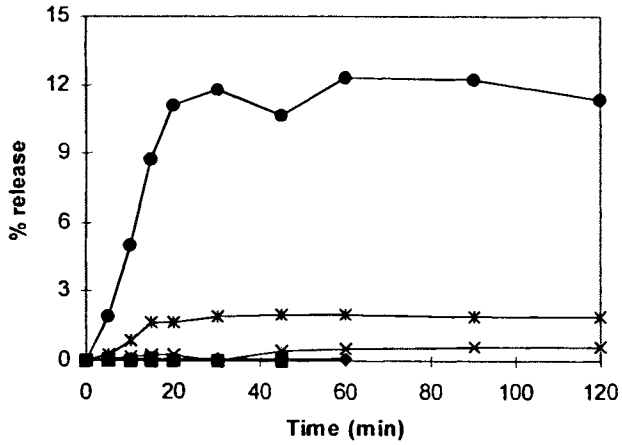

Fig. 2. Mean dissolution profiles of atovaquone from Wellvone® tablets (lot# C3377A) in various media. -◆- water; -■- SIF_{sp} (Athens); -▲- SIF_{sp} (Frankfurt); -✕- FaSSIF; -✳- FeSSIF; -●- milk.

of atovaquone in water or SIF_{sp} is practically nonexistent. The presence of bile components increased the dissolution of this compound, leading to an average maximum of 0.62% and 1.98% in FaSSIF and FeSSIF, respectively. As expected from its high lipophilicity, the extent of dissolution in milk was manifold higher than in all other media and reached a mean maximum of about 12%.

Based on the data shown in Fig. 2, one would guess that atovaquone is far from completely bioavailable from this type of formulation. It has been shown that, in the fed state, the absolute bioavailability of Mepron® tablets (atovaquone tablets equivalent to Wellvone® tablets) in HIV seropositive volunteers is about 21% (GlaxoWellcome data on file).

By comparing the milk vs. the water data (gastric conditions) and the FeSSIF vs. the FaSSIF data (intestinal conditions) it can be also concluded that the oral bioavailability of atovaquone from this product will be substantially higher in the fed state. The positive effects of food and bile on atovaquone absorption have been previously shown with a series of pharmacokinetic studies (14). Administration of 500 mg atovaquone in a tablet form 45 min. after a meal resulted in an increase in the geometric mean area under the concentration-time curve to infinity (AUC) of 1.2 to 3.9 times and in the C_{max} of 1.2 to 5.6 times, depending on the fat content of the co-administered meal (14). However, following an i.v. infusion of cholecystokinin octapeptide (which decreased gallbladder volume by 82% compared to the infusion of saline), administration of atovaquone tablets in the fasted state resulted in C_{max} values only 1.5 times higher (14). This finding suggests that dietary fats either enhance the solubilization effects of bile salts/lecithin micelles or directly dissolve the drug and is in accordance with the data shown in Fig. 2.

Sanfetrinem Cilexetil

Figure 3 shows the mean dissolution profiles of the two tested products. For the 630/C078/49 product, CVs were not higher than 11%, except for milk where CV values were as high as 48% at early time points. For the 630/C091/59 product, CVs reached a maximum of 52% at 5 minutes but were less than 26% at later time points. Dissolution of both products was greater at higher bile salt and lecithin concentrations (FeSSIF vs. FaSSIF vs. SIF_{sp}). Results in milk indicate that solubilization of this compound by the lipid and/or protein components was substantial, leading to plateau values of more than 70% within two hours for both products (Fig. 3).

The 630/C091/59 product is a standard wet-granulation formulation, while the 630/C078/49 product was prepared using a special wet-mixing method intended to increase dissolution and potentially the absorption of the compound. The excipients used in the two formulations were also quite different. However, results in milk, FaSSIF, and FeSSIF suggest that the oral bioavailability of the two products will be similar. The same *in vitro* data also suggest that the oral bioavailability of both products will probably be better at fed state. These predictions concurred with the results of two separate *in vivo* studies. The first study (crossover, 8 healthy volunteers) showed that in the fasted state the geometric mean C_{max} and AUC values of sanfetrinem were 1.2 μg/ml and 3.0 (μg/ml)h for the 630/C078/49 product and 1.4 μg/ml and 3.2 (μg/ml)h for the 630/C091/59 product, respectively (GlaxoWellcome data on file). The

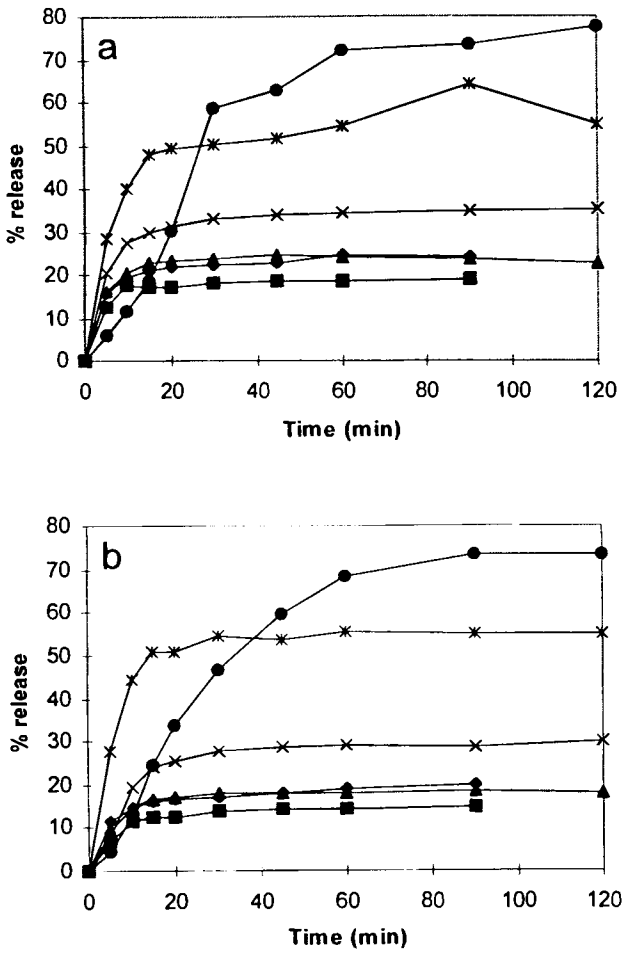

Fig. 3. Mean dissolution profiles of Sanfetrinem cilexetil in various media from two different IR tablets. (a) formulation 630/C078/49; (b) formulation 630/C091/59. -◆- water; -■- SIF_{sp} (Athens); -▲- SIF_{sp} (Frankfurt); -✕- FaSSIF; -✳- FeSSIF; -●- milk.

second study (crossover, fasted vs. fed state, two phases, 16 healthy volunteers) showed that, when the 630/C091/59 product was administered 30 min. after the completion of a meal, the geometric mean C_{max} value of sanfetrinem was 1.57 μg/ml. This value was significantly higher than the corresponding value in the fasted state (0.96 μg/ml) (GlaxoWellcome data on file).

GV150013X

Figure 4 shows the mean dissolution profiles of GV150013X in various media. CVs were as high as 22% at 5 min. but less than 13% after the 15 min. sample. Dissolution was greater at higher bile salt and lecithin concentration (FeSSIF vs. FaSSIF vs. SIF_{sp}). Because of the high log P value, one would expect much faster and more complete dissolution of GV150013X in FeSSIF than in FaSSIF. In the tested product, however, the active ingredient is co-spray dried with a polymer and is present in an amorphous, stable form (i.e., no reversion occurs) (GlaxoWellcome data on file). Therefore, dissolution is accelerated by the large, high energy surface area and lack of wetting problems. Thus, differences in dissolution between FaSSIF and FeSSIF medium were modest. The same arguments explain the profile in milk which, in contrast to atovaquone (a

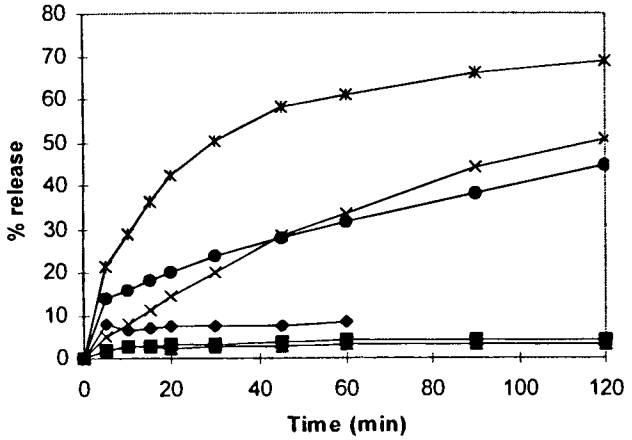

Fig. 4. Mean dissolution profiles of GV150013X from IR tablets (lot# M95/105A) in various media. -◆- water; -■- SIF_{sp} (Athens); -▲- SIF_{sp} (Frankfurt); -✕- FaSSIF; -✳- FeSSIF; -●- milk.

compound with log P similar to GV150013X), was similar to the profile in FaSSIF during the first two hours. It should be mentioned, however, that the maximum % dissolved in milk was 83% and was reached after approximately 10 hours (data not shown).

The dissolution data (Fig. 4) predict an increased oral bioavailability of the tested product when administered in the fed state, mostly due to enhanced gastric dissolution. Results from an *in vivo* study (crossover, 12 healthy volunteers) showed that geometric mean AUC value at fed state [27.6 (ng/ml)h] was significantly higher than the corresponding value at fasted state [20.1 (ng/ml)h] (GlaxoWellcome data on file).

DISCUSSION

Compendial dissolution media are primarily designed for quality control purposes and cannot be expected to be suitable in all cases for *in vitro/in vivo* correlations. In particular, food effects on drug absorption cannot be forecasted since there is no distinction made between fasted and fed state in the design of the compendial media. In contrast to the compendial media, the biorelevant media used in this study were specifically designed to simulate conditions in the upper GI tract in the fed and fasted state, and should, therefore, be capable not only of distinguishing between formulations with different bioavailabilities but also of predicting food effects on drug absorption. The results obtained were very encouraging. In all cases, the biorelevant media were able to predict trends in the *in vivo* data for both formulations and food effects on an *a priori* basis.

In the current study, fasted stomach was simulated with water. In principle, simulating fasted state conditions in the stomach requires a lower pH and a reduction of the surface tension (15). However, a lowering of the pH would be irrelevant to the dissolution of the four tested compounds since three are neutral and the fourth is a weak acid with extremely slow dissolution even at neutral pH.

Composition of gastric components in the fed state is highly dependent on the ingested meal and therefore it is difficult to design a medium which would be universally applicable. Milk is a logical starting point, in that it contains ratios of fat:protein:carbohydrate similar to those in a typical western

diet (16). Solubilization of drugs in milk is due to non-specific binding to casein micelles and, for highly lipophilic compounds, partitioning into the lipid components (10). Therefore, milk can be an appropriate medium to help assess differences between bile salt/lecithin solubilization and solubilization by dietary components. However, both casein micelles and lipids are partly digested during gastric residence (17–19). Therefore, although *in vitro* dissolution data in milk probably provide a better picture of gastric dissolution at fed state than data in the compendial simulated gastric fluid or other media simulating gastric composition in the fasted state (15), addition of physiologically relevant amounts of pepsin (and, perhaps, lipase) may be required to produce a closer simulation. Another issue associated with the use of milk relates to its variable composition from batch to batch, which in turn can induce variability in the *in vitro* dissolution data, especially for highly lipophilic compounds. Alternative media with more consistent composition may be appropriate in these cases. For the small intestine, FaSSIF is an approximate simulation of the average intralumenal composition and appeared to perform satisfactorily.

The medium used to represent fed state conditions in the small intestine, FeSSIF, is based on average pH and bile component concentrations in the fed state and does not account for the presence of ingested lipids. This may lead to underestimates of fed state dissolution in some cases for highly lipophilic compounds. Although forecasts of the effects of food on oral absorption were successful in the present study, one should keep in mind that in the food studies of troglitazone, atovaquone and sanfetrinem cilexetil products, the dosage form was administered 30 or 45 min after the completion of the meal. It is known that lumenal bile salt concentrations begin to fall towards the end of the first hour after food administration (e.g., 2). The concentration of bile salts/lecithin in FeSSIF is based on average peak values and may have, in the case of these particular studies (in which the drug was administered after peak bile output), offset the lack of dietary lipids in the medium. In addition, the results in both milk and FeSSIF need to be considered in order to arrive at a comprehensive picture of dissolution in the fed state.

A key difference between studies with biorelevant and compendial media is that, in the case of the biorelevant media, no attempt is made to adjust the composition to obtain sink conditions. In the case of class II compounds, sink conditions in the *in vitro* test are apt to be the exception rather than the rule, especially when a closed system apparatus (e.g., USP II) is used. For simulation of dissolution in the stomach this does not represent a problem, since absorption across the gastric mucosa is usually negligible. In the case of highly permeable drugs, it is often argued that sink conditions exist in the small intestine due to rapid removal of the drug from the lumenal fluid via absorption. Calculation of Dose to Solubility ratios (D:S) for the drugs studied (using the maximum concentrations reached in the various dissolution media as a first approximation for solubility) indicated that D:S is 3 to 6 liters in FaSSIF for the three troglitazone products, 80 liters for atovaquone, 1.4–1.7 liters for sanfetrinem cilexetil and one liter for GV150013X. In FeSSIF the values are 0.7–0.8, 25, 0.9, and 0.7 liters, respectively. Typical volumes available in the small intestine after meals range between one and 1.5 liters (20). In the fasted state, volumes are smaller due to the lower volumes ingested (only coadministered fluids) and secreted. Comparing these values with D:S, it appears that volumes required to maintain sink conditions will not exist in either the fasted or the fed state for any of the tested compounds. Therefore, only when absorption is extremely fast would the concentration in solution be kept low enough to maintain sink conditions. In such a case, data from a closed system apparatus can be useful for qualitative forecasts (e.g., rank order of formulations, qualitative prediction of food effects), but on a quantitative basis may underestimate the dissolution rate *in vivo*.

CONCLUSIONS

These studies demonstrate the utility of biorelevant media for *a priori* prediction of *in vivo* absorption of poorly soluble drugs. Not only trends in bioavailability with formulation but also food effects on bioavailability can be at least qualitatively predicted. For the four drugs studied, formulation effects were modest in comparison with food effects on absorption. Dissolution studies indicated that gastric dissolution (results in milk) as well as solubilization by bile components in the small intestine (FeSSIF results) may contribute to the enhanced bioavailability in the fed state. Although biorelevant dissolution tests still have issues which will require further refinement, they offer nonetheless a promising *in vitro* tool for P redicting the *in vivo* performance of drugs with dissolution limited absorption.

ACKNOWLEDGMENTS

This study was presented in part at the 12th and 13th annual meetings of the American Association of Pharmaceutical Scientists and it was sponsored by GlaxoWellcome R&D, UK. The authors would like to thank Dr. John Hempenstall (from GlaxoWellcome R&D, UK), Dr. Malcolm Young (from GlaxoWellcome Inc., North Carolina) and Drs Piero De Filippis and Lucio Da Ros (from GlaxoWellcome S.p.A., Italy) for their help in providing the *in vivo* and physicochemical data of the tested compounds.

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