

Introduction

Among the criteria required of compounds advancing from drug discovery programs, adequate systemic exposure (plasma concentrations or AUC) after oral dosing in preclinical models is one of the most important and sometimes most difficult to attain. Maximum oral bioavailability is desirable for both clinical and economic reasons. Low oral bioavailability is associated with high inter-subject variability of plasma concentrations, and therefore poor control of the drug's activities. And if bioavailability is low, much of the drug material is wasted, resulting in a certain economic disadvantage for costly drug substances.

Incomplete oral bioavailability could be due to poor solubility, poor intestinal permeability, or presystemic metabolism. It is not at all unusual for the majority of compounds in a drug discovery program to have poor water solubility. For example, Lipinski estimated that about one third of all new Pfizer (Groton) compounds have solubility ≤ 5 $\mu\text{g/mL}$ (1). Poor solubility is the one cause of incomplete oral bioavailability that can often be surmounted through formulation efforts. The optimization of oral bioavailability now usually begins early in the compound design and selection process, and in many pharmaceutical companies includes consideration of the influence of formulation on bioavailability. Productive drug discovery teams understand the limitations of poor water solubility, and know how to overcome these limitations.

One of the most important studies used to compare active compounds within a discovery program is the initial pharmacokinetic study, which is typically performed in rats or mice. Because pharmacokinetic studies require both animal and analytical resources, and because the PK data are so critical for compound selection, these studies may become a bottleneck in the compound screening process. If a new compound in a series shows poor pharmacokinetic properties in the initial study, it is not likely to be re-tested or advanced. To avoid discarding developable compounds having the desired pharmacologic properties, it is prudent to give each compound the best chance for success in the initial PK study. For poorly water soluble compounds this may involve the use of a potentially enabling formulation. For maximum oral absorption of poorly soluble compounds it is necessary to solubilize the compound and keep it in solution in the gastrointestinal tract.

We have used a tiered approach that includes *in vitro* solubility assays with minimum compound requirements to identify dosing vehicles that might be most useful for oral delivery of our Sponsors' compounds. Here we describe this tiered approach to selecting dosing formulations for preclinical pharmacokinetic studies. We also provide examples of the application of this tiered approach for two poorly water soluble compounds already available as marketed drugs.

A Tiered Approach for Identifying Enabling Formulations

We believe that formulation efforts at the discovery stage should have the following objectives.

- To give developable compounds the best chance for success in the initial preclinical pharmacokinetic study.
- To use the simplest formulation as possible.

The overall aim is to balance what it takes to get the compound absorbed, with consideration that the more heroic the dosing formulation is, the more difficult it may be for further development. If a compound is accepted for advancement to development based on PK results obtained using an unconventional formulation, there must be a feasible way to formulate that compound for safety studies and clinical trials.

A tiered formulation approach that can be used to evaluate poorly soluble compounds is summarized below. This table also lists some of the factors that will need to be considered later in development if this formulation approach is used.

Table 1.

| Tier | Formulation Components | Factors that will be Important Downstream |
|------|--|--|
| 1 | Primarily aqueous solutions and suspensions with pH adjustment | Least formulation development issues |
| 2 | Primarily aqueous with solubilizing agents <ul style="list-style-type: none"> - Cyclodextrins - Surfactants such as polysorbate 80, Cremophor, Soluene, vitamin E TPGS, poloxamers - Povidone - Dimethyl Isosorbide - Low concentrations of the solvents listed in Tier 3 | Acceptability and cost of solubilizing agents |
| 3 | Primarily cosolvent (+/- other solubilizers) <ul style="list-style-type: none"> - PEG 300, 400 - Propylene glycol - DMSO - DMA - Ethanol - N-methylpyrrolidone - Trimethylglycol | Above plus... Drug load and dose/tolerability limits of cosolvent Chemical and physical stability Is development of solid formulation possible? |
| 4 | Lipid based (+/- surfactants) <ul style="list-style-type: none"> - Oils - Medium chain glycerides - Labrafil - Labrasol - Gelucires | Above plus... Formulation options may be restricted |

Cremophor (BASF) is a group of polyoxyl hydrogenated castor oil excipients.

Solutol (BASF) is PEG15 hydroxystearate.

Labrafil, Labrasol, and Gelucire (Gattefosse) are mixtures of glycerides and PEG esters.

Tier 1 is the simplest formulations such as aqueous solutions and suspensions. If Tier 1 discovery formulations provide adequate oral absorption, adequate oral bioavailability should also be readily attainable with conventional solid dosage forms.

Tier 2 formulations utilize an added level of complexity, the use of a solubilizing agent in a primarily aqueous dosing formulation. Compounds with adequate oral absorption using Tier 2 discovery formulations may also eventually achieve adequate oral bioavailability with conventional solid dosage forms. However, if Tier 2 dosing formulations are used in preclinical safety studies, the acceptability of the solubilizing agent will be an important consideration.

Tier 3 discovery formulations are primarily non-aqueous. Some of the non-aqueous solvents that are acceptable for animal studies are listed in Table 1. Most of these, including DMSO, DMA, and ethanol, have limits for the percentage that can be dosed and the total amount that can be dosed. Therefore, Tier 3 formulations often are comprised of combinations of co-solvents. Compounds that attain adequate oral absorption with Tier 3 discovery dosing formulations may require more formulation development work than other compounds to achieve acceptable formulations for safety studies, clinical trials, and drug product.

Tier 4 discovery formulations are comprised primarily of lipid components. These may be particularly effective in aiding the oral absorption of poorly water soluble compounds through their emulsifying activity. However, if only Tier 4 formulations are successful in achieving bioavailability, subsequent formulation development options may be limited.

For each Tier of the discovery formulation approach there is a balance between the extent of oral absorption achievable and the possible limitations of the formulation at a later stage. The acceptability of any particular formulation approach will be different for each program, and possibly among different compounds within a program, and will vary from company to company.

To illustrate the use of the tiered formulation approach, we performed *in vitro* formulation evaluation and solubility studies to select dosing vehicles for rat pharmacokinetic studies with two poorly water soluble drugs, carbamazepine and itraconazole. We felt it would be useful to test some poorly soluble marketed drugs for which the feasibility of development is not in question. Some properties of these compounds are listed below.

Table 2.

| | Carbamazepine | Itraconazole |
|---------------------------------|----------------------|--|
| Aqueous solubility | 158 µg/mL | <3.5 µg/mL |
| Molecular weight | 236 | 706 |
| clog P | 2.38 | 5.66 |
| Available products | 200 mg tablets | 100 mg capsule, 10 mg/mL solution (with hydroxypropyl-β-cyclodextrin) |
| Absorption in humans (F) | >70% | 55% |

Methods

Dosing formulations were selected through a preliminary examination of various potential formulations beginning with Tier 1 and progressing through Tiers 2 and 3, using pharmaceutically acceptable components and concentrations generally recognized as safe for preclinical use. The assessment of suitability for *in vivo* dosing was based on appearance (solubilization, viscosity) and conformance with QPS IACUC guidelines, as well as solubility after aqueous dilution. If the compound was not completely soluble in a vehicle used for dosing, aliquots of the dosing formulation were taken after filtration and analyzed to provide a measure of solubility in the dosing vehicle.

Male Sprague Dawley rats weighing approximately 225-250 g were used in this study. Rats were obtained from Hilltop Research, and each had a jugular vein cannula (JVC) implanted for blood sample collections. Some rats were administered the test compound intravenously (IV), and these rats also each had a femoral vein cannula implanted for IV dose administration. There were 3 animals per dose group, and animals were fasted overnight before dosing until 4 hours post-dose. The doses used in this study were 1 mg/kg IV and 10 mg/kg PO. The dose volumes were 1 mL/kg for IV and PO, although itraconazole was also tested with some oral formulations using a dose volume of 10 mL/kg. The PO doses were administered by gavage. The IV doses were administered through the femoral vein cannula as a slow (<1 minute) bolus. Blood samples (0.5 mL) were collected at the following times:

- PO: Pre-dose, 15, 30 min, 1, 2, 4, 6, 8 and 24 hr post-dose
- IV: Pre-dose, 5, 15, 30 min, 1, 2, 4, 6, 8 and 24 hr post-dose

The whole blood was collected into tubes containing K₂EDTA as an anticoagulant on wet ice and spun down within 60 min (at 4°C). Plasma (approximately 0.2 mL) was transferred to labeled tubes and stored at -20°C until analysis. Blood removed as specimens was replaced by blood transfusion using fresh blank rat blood.

Sample analysis was performed using LC/MS/MS with internal standard after protein precipitation of plasma. Duplicate standard curves were run before and after samples. Calibration curves included double blank, single blank (Internal Standard only), and a minimum of 5 standards with LLOQ targeting approximately 1 ng/mL, prepared in blank rat plasma. Dosing formulation aliquots were analyzed after appropriate dilution in rat plasma. Non-compartmental pharmacokinetic analysis of the plasma concentrations vs. time data was performed using WinNonlin™ (Pharsight Corporation).

Results and Discussion

Potential dosing vehicles were evaluated with the objectives of getting the compound in solution and keeping it in solution when diluted into an aqueous environment. Using the tiered approach, we started with the simplest dosing vehicles (Tier 1), and advanced through Tier 3 for these two test compounds. Tier 4 (lipid based) formulations were not evaluated for these compounds because it was already known that adequate absorption of these compounds should be attainable without the use of excipients in this category. One or more dosing formulation from each of Tiers 1, 2, and 3 was evaluated *in vivo* for each compound, so as to assess the impact of formulation on oral absorption. The *in vitro* assessment of various dosing formulations for each compound is summarized in Tables 1 and 2. The formulation assessment studies were performed using a few mg to prepare each potential formulation evaluated. Minimization of compound usage is very important at the discovery stage, when little compound is generally available. Compound usage can be further minimized by preparing a single concentrated stock solution in a solvent that can be dosed at a low concentration, such as dimethylsulfoxide (DMSO) or dimethylacetamide (DMA).

To determine absolute oral bioavailability, each test compound was also administered IV. For carbamazepine, the IV dosing formulation was 10 mg/mL in 40% hydroxypropyl- β -cyclodextrin (HP β CD) in water. For itraconazole, the IV dosing formulation was 10 mg/mL in 10% dimethylacetamide, 60% PEG 400, 30% of 40% HP β CD in water. These dosing vehicles were identified based on the testing of oral formulations.

Carbamazepine

Pharmacokinetic results for carbamazepine are given in Figure 1 and the attached table. As summarized in Table 3, carbamazepine was not completely soluble in the Tier 1 formulations tested, but was soluble in the Tier 2 formulations. Carbamazepine remained in solution when Tier 2 and Tier 3 formulations were diluted 50X with water. The measured concentration in a filtrate of the 1% methylcellulose suspension formulation was 0.06 mg/mL, which is slightly lower than the reported aqueous solubility. The methylcellulose suspension formulation provided 50% oral bioavailability. When carbamazepine was dosed using the Tier 2 (40% HP β CD) or Tier 3 (DMA/PEG) dosing solutions, bioavailability was complete. Figure 2 shows individual plasma carbamazepine concentration vs. time profiles for the Tier 1 and 2 formulations. The suspension resulted in a delayed absorption profile, particularly in 2 of the 3 rats, compared with the solution formulation. Carbamazepine represents an example of a compound for which formulation influences bioavailability, but it has sufficient solubility such that acceptable properties in a discovery PK study would be obtained using the most basic formulation. The formulation and PK results we obtained are consistent with the hypothesis stating that getting the compound in solution and keeping it in solution can maximize oral bioavailability.

Table 3. Carbamazepine Oral Formulation Evaluation

| | Composition | Observation 1 | Observation 2 | Pathforward |
|--------|---|---|---------------------------------------|--|
| Tier 1 | 10 mg/mL in 1% methylcellulose in water | Not completely soluble (Measured solubility = 0.06 mg/mL) | | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |
| | 1 mg/mL in 1% methylcellulose | Not completely soluble | | |
| Tier 2 | 10 mg/mL in 40% hydroxypropyl- β -cyclodextrin (HP β CD) in water | Soluble after sonication | Soluble after diluting 50X with water | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |
| Tier 3 | 10 mg/mL in 10% DMA / 90% PEG 400 | Soluble | Soluble after diluting 50X with water | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |

Figure 1. Mean (+SD) Plasma concentration vs. time profiles and pharmacokinetic properties for carbamazepine in rats after 1 mg/kg IV or 10 mg/kg PO doses in various formulations.

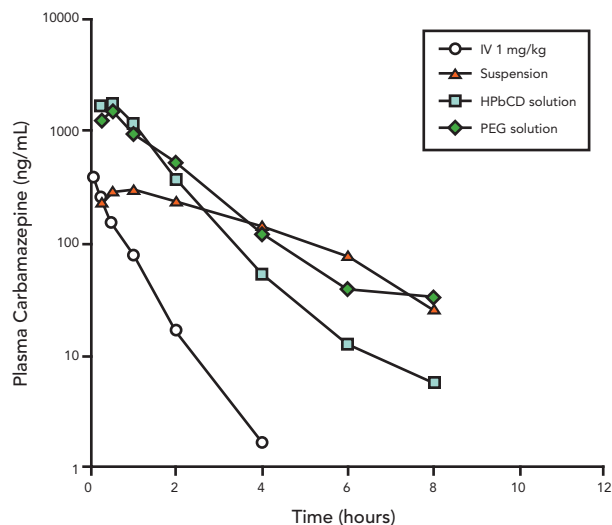
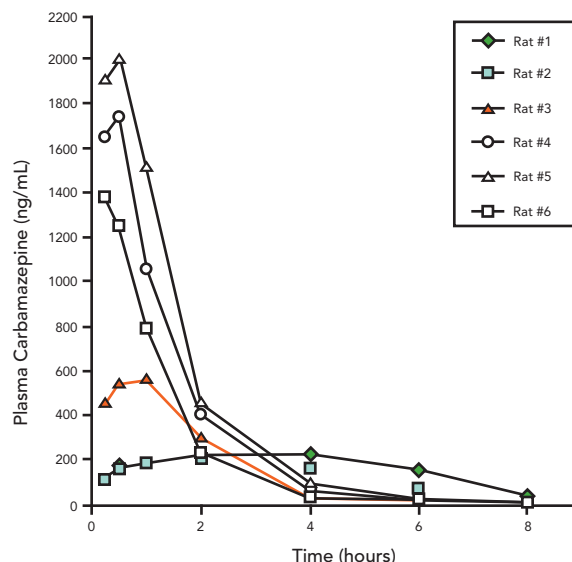


Figure 2. Plasma concentration vs. time profiles for carbamazepine in individual rats administered suspension (Rats #1, 2, 3) or HPbCD solution (Rats #4, 5, 6) formulations.



| | Suspension | | HPbCD solution | | PEG solution | |
|------------------------|------------|-----|----------------|-----|--------------|-----|
| | Mean | SD | Mean | SD | Mean | SD |
| 10 mg/kg PO | | | | | | |
| C_{max} (ng/mL) | 332 | 195 | 1704 | 315 | 1467 | 583 |
| T_{max} (h) | 2.3 | 1.5 | 0.4 | 0.1 | 0.5 | 0.0 |
| AUC_{last} (ng*h/mL) | 1219 | 174 | 2561 | 725 | 2761 | 177 |
| $t_{1/2}$ (h) | 1.6 | ND | 1.5 | 0.5 | 1.5 | 0.5 |
| AUC_{inf} (ng*h/mL) | 1183 | 120 | 2573 | 721 | 2857 | 116 |
| F (%) | 50 | 6 | 104 | 29 | 116 | 5 |

ND: Not determined

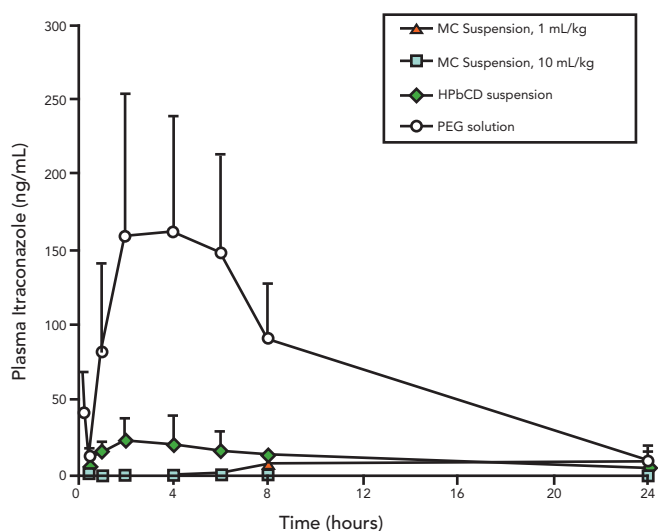
Itraconazole

Itraconazole could be considered a more challenging compound to deliver orally, consistent with its lower solubility. The *in vitro* formulation assessment results shown in Table 4 indicate that it was not soluble in the Tier 1 or Tier 2 formulations tested, and a Tier 3 formulation was required for complete solubilization. Pharmacokinetic results for oral itraconazole are summarized in Figure 3 and its attached table. A methylcellulose suspension formulation (Tier 1) was dosed at both 10 mg/mL and 1 mg/mL concentrations for 10 mg/kg doses, but both resulted in variable and low oral absorption. The Tier 2, HPbCD formulation was dosed as a suspension and resulted in improvement of oral bioavailability to $15 \pm 7\%$. The Tier 3 formulation improved oral bioavailability further to $56 \pm 21\%$. It should be noted that this formulation resulted in itraconazole precipitation when diluted 50X with water. Further formulation optimization work might enable even greater itraconazole absorption in this preclinical model. Itraconazole represents an example of a compound for which formulation not only influences bioavailability, but could have been critical to the identification of this as a developable compound.

Table 4. Itraconazole Oral Formulation Evaluation

| | Composition | Observation 1 | Observation 2 | Pathforward |
|---------------|---|---|--|---|
| Tier 1 | 10 mg/mL in 1% methylcellulose in water | Not completely soluble (Measured solubility <0.001 mg/mL) | | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |
| | 1 mg/mL in 1% methylcellulose | Not completely soluble (Measured solubility <0.001 mg/mL) | | Dosed <i>in vivo</i> at 10 mL/kg (10 mg/kg) |
| Tier 2 | 10 mg/mL in 40% hydroxypropyl-β-cyclodextrin (HPβCD) in water | Not completely soluble (Measured solubility = 0.25 mg/mL) | | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |
| | 1 mg/mL in 40% hydroxypropyl-β-cyclodextrin (HPβCD) in water | Not completely soluble | | |
| | 10 mg/mL in 10% dimethylacetamide (DMA) & 40% HPβCD in water | Not completely soluble | | |
| | 10 mg/mL in 10% dimethylacetamide (DMA) & 30% Solutol HS15 in water | Not completely soluble | | |
| Tier 3 | 10 mg/mL in 10% DMA / 89% PEG 400 / 1% polysorbate 80 | Soluble | Precipitates after diluting 50X with water | Dosed <i>in vivo</i> at 1 mL/kg (10 mg/kg) |

Figure 3. Mean (+SD) Plasma concentration vs. time profiles and pharmacokinetic properties for itraconazole in rats after 10 mg/kg PO doses in various formulations.



10 mg/kg PO

| | MC Suspension, 1 mL/kg | | MC Suspension, 10 mL/kg | | HPβCD suspension | | PEG solution | |
|-------------------------------|------------------------|------|-------------------------|-----|------------------|-----|--------------|-----|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| C _{max} (ng/mL) | 12 | 8 | 3 | 2 | 26 | 15 | 197 | 59 |
| T _{max} (h) | 18.0 | 10.4 | 2.9 | 4.4 | 4.7 | 3.1 | 3.3 | 2.3 |
| AUC _{last} (ng*h/mL) | 165 | 128 | 2 | 2 | 320 | 126 | 1830 | 657 |
| t _{1/2} (h) | 10.2 | ND | ND | ND | 24.3 | ND | 4.6 | ND |
| AUC _{0-∞} (ng*h/mL) | 53 | ND | ND | ND | 509 | 304 | 1854 | ND |
| F (%) | 5 | 4 | <1 | <1 | 15 | 7 | 56 | 21 |

ND: Not determined

Conclusions

Here we propose a tiered approach for assessing discovery stage formulations and identifying the simplest formulations that can maximize oral absorption of poorly water soluble compounds. These formulation identification and pharmacokinetic studies provide an understanding of the formulation requirements for achieving oral absorption. This was illustrated with carbamazepine, which had reasonable bioavailability with a simple methylcellulose suspension, and itraconazole, a more poorly soluble compound which required more complicated formulations to achieve oral bioavailability.

References

- 1) C. Lipinski, Current Drug Discovery, April 2001.
- 2) Zhou *et al.*, J. Pharm. Sci. 96, 3052 (2007).
- 3) Goodman & Gilman's The Pharmacological Basis of Therapeutics (1996).



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