Rapid In Vivo Oral Screening in Rats: Reliability, Acceptance Criteria, and Filtering Efficiency

Submitted: August 11, 2005; Accepted: February 17, 2006; Published: July 28, 2006

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ABSTRACT

The reliability and acceptance criteria of rapid oral exposure screening were evaluated by pharmacokinetic simulations and by comparing oral exposure of 100 proprietary compounds from 15 therapeutic programs obtained at different times by cassette accelerated rapid rat screen (CARRS) and conventional pharmacokinetic (full-PK) procedures. Once acceptance criteria were established, the filtering efficiency (discard rate) was assessed with a larger data set of 5289 compounds tested by CARRS only. These evaluations indicated that area under the concentration-time curve during the first 6 hours (AUC_{6h}) captured >50% of AUC_∞ for most (71%) of the compounds and AUC_{6h} from CARRS is comparable to AUC_{6h} from full-PK in categorizing oral exposure as low, moderate, or high; therefore, the truncated AUC_{6h} derived from pooled plasma samples is suitable for oral exposure screening. The CARRS profiles did not provide reliable half-life estimates; however, compounds with substantial AUC beyond 6 hours can be identified when ($C_{6h}/C_{max} \times 100\%$) exceeds 80%. Of interest, both the observed data and the simulated data indicated that AUC_{6h} can be estimated using a single time point plasma concentration at 3 hours. The relationship between the maximum bioavailability and AUC_∞ over a range of clearance values was simulated. A threshold AUC (500 h*ng/mL) at the routine screening dose of 10 mg/kg was established below which a compound can be discarded. Examination of screening results for 5289 compounds evaluated over the last few years in our laboratory indicated that CARRS had a filtering efficiency of 50%, suggesting that this criterion provides a useful decision gate to avoid wasting the drug discovery resources on nonviable candidates.

KEYWORDS: oral exposure, pharmacokinetics, rat, screen

INTRODUCTION

In order to accelerate the drug discovery process, various in silico, in vitro, and in vivo high-throughput screening procedures have been developed to assess pharmacokinetic (PK) properties for biologically active compounds.[1,2] Although more resource and labor intensive than in vitro experiments, animal studies are considered to be the most predictive of human PK. In addition, obtaining acceptable PK in rats can be important for proof-of-concept in rat disease models and for the successful conduct of a general toxicology program. High-throughput in vivo methods are principally achieved by minimizing the time and labor spent in the animal experiment, bioanalytical analysis, and report preparation. Cassette dosing or "N-in-one" dosing[3,4] and sample pooling[5-8] are recent examples that exploit liquid chromatography-tandem mass spectrometry (LC-MS/MS) for high-throughput in vivo screening. Because of the potential for drug-drug interactions and other disadvantages of cassette dosing,[9] a "rapid rat" PK screening procedure, in which compounds are dosed to individual rats, was developed and automated as described elsewhere.[10,11] The fast turnaround time is achieved by batch processing (referred to as cassette accelerated rapid rat screen, CARRS) with simplified and standardized procedures such as pooled samples, sparse sampling, 3-point standard curve, automated sample preparation, LC-MS/MS analysis, and report templates.[11] Because this screening approach uses pooled plasma samples and a truncated PK curve (up to 6 hours only), the primary objectives of the current evaluation were to (1) determine if these screening "shortcuts" negatively affect the quality of data and the screening outcomes, and (2) propose an oral AUC threshold that can be used for decision making purposes. A secondary objective was to determine if any additional PK parameters would add value to the interpretation of the oral (PO) screening results.

METHODS

Data Collection

One hundred compounds from 15 different discovery programs, which had been tested by both CARRS and full PK, were selected for this evaluation. The molecular weights ranged from 266 to 791 with a median at 514. Since these studies occurred on different occasions, in many cases they involved 2 different chemical batches. In most cases, amorphous material was used for both CARRS and full-PK studies, and compounds were prepared as 0.4% methylcellulose suspensions for PO dosing and as hydroxypropyl B-cyclodextrin (HPβCD) solutions for intravenous (IV) dosing.

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Data from 5289 compounds screened with CARRS during the last 2 years were used for the evaluation of filtering efficiency (discard rate).

PK Calculation of CARRS and Full-PK Studies

For CARRS, each compound was individually dosed at 10 mg/kg to 2 rats. Six samples were obtained at 0.5, 1, 2, 3, 4, and 6 hours and pooled from each rat at the identical time points. For the full-PK studies, rats were dosed IV and PO (3 rats each route; dose range of 1-10 mg/kg) and ~8 to 10 plasma samples were collected over 24 hours. All plasma samples were analyzed individually for parent drug by LC-MS/MS.[10,11] For CARRS, area under the concentration-time curve during the first 6 hours (AUC_{6h}) was estimated by the trapezoidal rule. For full-PK studies, standard PK parameters such as area under the concentration curve AUC_{∞}, volume of distribution at the steady-state (Vd_{ss}), mean residence time (MRT), half-life ($t_{1/2}$), systemic clearance (CL), and oral bioavailability (F) were obtained using noncompartmental analysis (Watson LIMS, Innaphase, Philadelphia, PA).

Simulations

Simulations were performed to find out the theoretical relationship between ($AUC_{6h}/AUC_{\infty} \times 100\%$) and ($C_{6h}/C_{max} \times 100\%$) and the relationship between AUC_{6h} and C_{3h} at a dose of 10 mg/kg in rats. Standard 1-compartment or 2-compartment models with elimination from the central compartment were used.[12] The selected ranges of PK parameters for simulations were similar to the actual range observed in the 100-compound test set. For the 1-compartment model (Figure 1), plasma concentrations were simulated with Equation 1 using k_{01} from 0.2 to 1 $hour^{-1}$ and k_{10} from 0.035 to 0.69 $hour^{-1}$ ($t_{1/2}$ range of 1-20 hours) with a total of 72 combinations.

$$C(t) = \frac{FDK_{10}}{V(k_{01} - k_{10})}(e^{-k_{10}t} - e^{-k_{01}t}), \quad (1)$$

where C(t) is concentration at time t; F is bioavailability; D is dose; V is volume of distribution; k_{01} is absorption rate; mean absorption time (MAT) $=1/k_{01}$; k_{10} is elimination rate; and terminal half life ($t_{1/2,}\beta$) $=0.693/k_{10.}$ For the 2-compartment model (Figure 2), Equation 2 was used.

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + C^{-k_{01}t}, \quad (2)$$

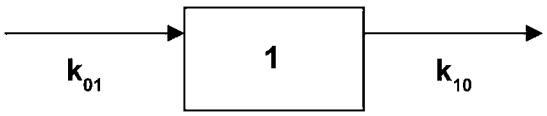

Figure 1. Model scheme for 1-compartment model with first-order absorption and first-order elimination.

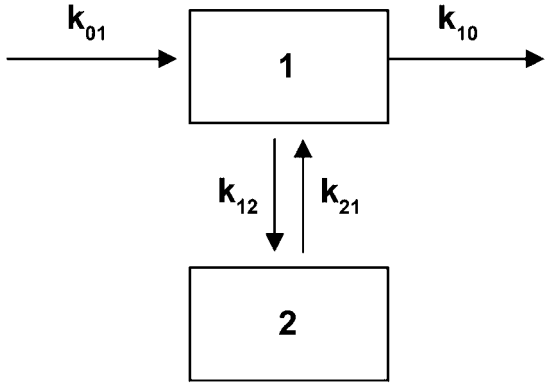

Figure 2. Model scheme for 2-compartment model with first-order absorption and first-order elimination from central compartment.

where $A = \frac{\frac{FD}{V}k_{01}(k_{21} - \alpha)}{(\alpha - \beta)(\alpha - k_{01})}$ $B = \frac{-\frac{FD}{V}k_{01}(k_{21} - \beta)}{(\alpha - \beta)(\beta - k_{01})}$ $C = \frac{\frac{FD}{V}k_{01}(k_{21} - k_{01})}{(\beta - k_{01})(\alpha - k_{01})}$ and α and β ($\alpha > \beta$) are roots of the quadratic equation. k_{01} is absorption rate; MAT $=1/k_{01}$; k_{10} is elimination rate for central compartment; $t_{1/2,k10} = 0.693/k_{10}$; $t_{1/2,}\beta = 0.693/\beta$; k_{12} is transfer rate for compartment 1 to 2; k_{21} is transfer rate for compartment 2 to 1. Concentrations were simulated under 2 situations (1) $k_{21} >> k_{12}$ and (2) $k_{12} >> k_{21}$. For situations with $k_{21} >> k_{12}$, the simulation was performed with k_{21} fixed at 0.69 $hour^{-1}$ and k_{12} at 0.069 $hour^{-1}$; k_{01} varied from 0.2 to 1 $hour^{-1}$ and k_{10} varied from 0.039 to 1.4 $hour^{-1}$ (corresponding $t_{1/2,}\beta$ range of 1-20 hours) with 78 combinations. For situations with $k_{12} >> k_{21}$, k_{12} was fixed at 0.69 $hour^{-1}$ and k_{21} at 0.069 $hour^{-1}$, k_{01} varied from 0.2 to 1 hour, and k_{10} was selected from 0.77 to 35 $hours^{-1}$ (corresponding $t_{1/2,}\beta$ range of 10-19 hours), another 78 combinations. Random error was added to the calculated concentration values for each simulated condition, assuming a normal distribution of error with a 15% coefficient of variation (CV), using the function NORMINV(RAND(), MEAN, STDEV) in Microsoft Excel (Microsoft, Redmond, WA). All simulations assumed first-order drug absorption with an MAT range of 0.5 to 5 hours. The AUC was calculated with the simulated concentration-time profile using the trapezoidal rule. In the simulation of relationships of ($C_{6h}/C_{max} \times 100\%$) versus ($AUC_{6h}/AUC_{\infty} \times 100\%$), and C_{3h} versus AUC_{6h}, the term of FD/V cancels out; therefore, changes in F, D, and V have no effect on these relationships, thus F, D, and V were fixed at 1, 10 mg/kg, and 1000 mL/kg, respectively.

The relationship between F and AUC_{∞} was also simulated with the following assumptions: (1) compounds are equally distributed between blood cells and plasma ($C_{blood}/C_{plasma} = 1$), (2) elimination exclusively occurs by the liver, therefore total systemic clearance (CL) equals hepatic clearance (CL_H), and (3) F_A and F_H are the major factors that contribute to the bioavailability ($F = F_A * F_H$). F_A is the fraction of the dose absorbed into enterocytes that escapes presystemic

intestinal elimination, and F_H is the fraction of compound entering the liver that escapes presystemic hepatic elimination. At a dose of 10 mg/kg in rats, the relationship between F and the corresponding AUC_∞ can be obtained with the following equations:

$$F_H = 1 - \frac{CL_H}{Q_H} = 1 - \frac{CL}{Q_H} \quad (3)$$

$$AUC_\infty = F_A \times F_H \times \frac{\text{Dose}}{CL} = F_A \times \left(1 - \frac{CL}{Q_H}\right) \times \frac{\text{Dose}}{CL} \quad (4)$$

The mean value of liver blood flow (Q_H) of rats (65 mL/min/kg) was used.[13] The 95% confidence interval (CI) of the relationship between AUC and F was established by assuming a 30% CV for Q_H with 8 replicates for each situation corresponding to more than 100 liver extraction ratio (E_H) values ranging from 0.01 to 0.99 (F_A fixed at 1).

Results and Discussion

Distribution of Pharmacokinetic Parameters of Selected Compounds

The distribution of PK parameters for this series of compounds, including elimination rates ($t_{1/2}$ and MRT), CL, Vd_{ss}, F, MAT, and time of maximum concentration (T_{max}) are summarized in Table 1. As shown, most compounds had PK parameters that are in the range of values typically observed in rats during lead optimization. These compounds have CL values evenly distributed between 1 and 75 mL/min/kg and F values evenly distributed between 1% and 100%. Thus, even though these compounds progressed from screening to the full-PK studies, they did not show a skewed distribution toward more favorable PK parameters.

Is the Truncated AUC_{6h} Misleading for Screening?

A key factor that contributes to optimum resource utilization and fast turnaround time for oral exposure screening is shortening the sample collection time to 6 hours, which allows study initiation and completion to occur on the same day. Automated blood sampling systems, while ideal for reducing the human labor component and for collection of after-hours time points (eg, 12 and 24 hours) in PK studies, would not offer any productivity advantage to CARRS compared with the current manual system.

In order to evaluate whether truncating to 6 hours is acceptable for screening, ($AUC_{6h}/AUC_\infty \times 100\%$) was calculated using the conventional PK profiles (Table 2). For these 100 compounds, 71% had AUC_{6h} that captured the majority (>50%) of their corresponding AUC_∞. For the remaining 29 compounds that had significant AUC beyond 6 hours (ie, AUC_{6h} is substantially underestimating AUC_∞), only 7 compounds had AUC_{6h} values below the recommended AUC cutoff value of 500 hours*ng/mL (discussed later in What is a "Good" AUC from PO Screening Studies?) and would be classified as false negatives (see footnotes for Table 2). There are 27 compounds where AUC_{6h} substantially underestimates the AUC_∞ ($AUC_{6h}/AUC_\infty \times 100\% < 50\%$; however, AUC_{6h} of these compounds are well above the 500-hour*ng/mL cutoff, which would be considered as true positives. A more accurate AUC of these compounds will be determined in a more rigorous full-PK study subsequent to this screening result. Therefore, the overall true positive rate was 87% (60% + 27%), the true negative rate was 11%, the false positive rate was 0%, and the false negative rate was only 2% for CARRS. Therefore, AUC_{6h} generated by CARRS can be reliably used to discard compounds with poor oral exposure.

Can the Truncated Screening Profile Be Used to Estimate AUC Beyond 6 Hours?

If elimination $t_{1/2}$ estimates could be obtained from CARRS data, then AUC_∞ could be estimated from AUC_{6h} by standard

Table 1. The Distribution of PK Parameters of 100 Selected Compounds*

Values of Parameters	$t_{1/2}$ (hours)	MRT IV (hours)	MAT (hours)	T_{max} (hours)	CL (mL/min/kg)	Vd_{ss} (L/kg)	F%
<1	0	8	26	35	2	7	0
1-3	31	43	58	43	3	35	2
3-6	43	35	14	14	8	30	3
6-10	20	9	1	8	10	12	4
10-25	5	5	0	0	27	14	19
25-50	0	0	1	0	31	2	35
50-75	1	0	0	0	13	0	22
>75	0	0	0	0	6	0	15
Total	100	100	100	100	100	100	100

*$t_{1/2}$ indicates half-life; MRT, mean residence time; MAT, mean absorption time; T_{max}, time of maximum concentration; CL, systemic clearance, Vd_{ss} volume of distribution at the steady-state; and F, oral bioavailability.

Table 2. Distribution of Compounds Based on Percentage of AUC Captured in 6 hours and Distribution Relative to the AUC Threshold of 500 h*ng/mL*

	(AUC_{6h}/ AUC_{∞} *100%) <50%	(AUC_{6h}/ AUC_{∞} *100%) >50%	Total
Number of compounds	29	71	100
Number of compounds with AUC_{6h} > 500 h*ng/mL in full-PK	22 (true +)	60 (true +)	82
Number of compounds with AUC_{6h} < 500 h*ng/mL in full-PK	7 (false −)	11 (true −)	18
Number of compounds with AUC_{∞} > 500 h*ng/mL in full-PK	27 (true +)	63 (true +)	90
Number of compounds with AUC_{∞} < 500 h*ng/mL in full-PK	2 (false −)	8 (true −)	10
Number of compounds with AUC_{6h} > 500 h*ng/mL in CARRS	27 (true +)	60 (true +)	87
Number of compounds with AUC_{6h} < 500 h*ng/mL in CARRS	2 (false −)	11 (true −)	13

*AUC indicates area under the concentration curve; full-PK, conventional pharmacokinetic procedure; and CARRS, cassette accelerating rapid rat screen. An AUC_{∞} threshold of 500 h*ng/mL can be used to discard compounds with low oral bioavailability at a dose of 10 mg/kg in rats. Thus, the true positives are compounds in which both AUC_{6h} and AUC_{∞} > 500 h*ng/mL; the true negatives are compounds with AUC_{6h} or AUC_{∞} < 500 h*ng/mL; the false negatives are compounds with AUC_{6h} < 500 h*ng/mL, but AUC_{∞} > 500 h*ng/mL; There is no false positive category.

log-linear extrapolation. In the full-PK studies, most of the tested compounds (78%) had T_{max} < 3 hours, and 30% of the compounds had $t_{1/2} \leq 3$ hours; thus, it is theoretically possible that there might be sufficient postabsorption data points for $t_{1/2}$ estimation, even with the truncated 6-hour screening profile. However, after careful examination of $t_{1/2}$ values obtained from individual animals and those obtained from pooled samples after oral administration, it was found that $t_{1/2}$ estimates from pooled samples are artificially prolonged and potentially misleading. In addition, for 15% of these compounds, the pooling procedure created a flat profile up to 6 hours precluding $t_{1/2}$ estimation, whereas there was a clear downward slope within the 6-hour profile for these compounds in the full-PK studies (data not shown). The cause of this artificially prolonged $t_{1/2}$ of pooled samples is likely due to a decrease in time-dependent differences in concentration when pooling individual profiles that have different T_{max}.

Since $t_{1/2}$ estimates from CARRS profiles are unreliable, an alternative approach was tried to find a way to estimate the residual AUC beyond 6 hours based solely on the truncated 6-hour profile. The magnitude of the 6-hour concentration relative to the maximal concentration ($C_{6h}/C_{max} \times 100\%$) was highly correlated with the percentage of infinity area captured within the first 6 hours ($AUC_{6h}/AUC_{\infty} \times 100\%$) for both the observed data set ($r^2 = 0.77$; Figure 3A) and the simulated data set (Figure 3B). For example, for compounds with ($C_{6h}/C_{max} \times 100\%$) < 20% (ie, at least a 5-fold reduction between C_{max} and C_{6h}), the ($AUC_{6h}/AUC_{\infty} \times 100\%$) is usually greater than ~70%, indicating AUC_{6h} is a reasonable estimate of AUC_{∞}. On the other hand, for compounds with ($C_{6h}/C_{max} \times 100\%$) > 80% (ie, concentrations have fallen less than 20% between C_{max} and C_{6h}), AUC_{6h} is usually less than 30% of AUC_{∞}, indicating that AUC_{6h} is a

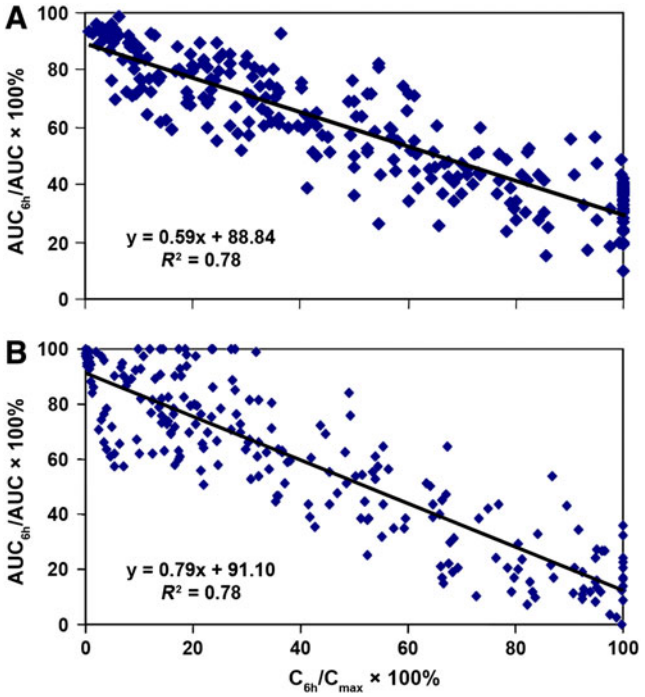

Figure 3. Relationship between ($AUC_{6h}/AUC_{\infty} \times 100\%$) and ($C_{6h}/C_{max} \times 100\%$): (A) correlation of ($AUC_{6h}/AUC_{\infty} \times 100\%$) and ($C_{6h}/C_{max} \times 100\%$) with 100 compounds in 254 rats; (B) correlation of ($AUC_{6h}/AUC_{\infty} \times 100\%$) and ($C_{6h}/C_{max} \times 100\%$) with simulated profiles using 1- and 2-compartment models with an MAT range of 0.5 to 5 hours, a terminal $t_{1/2}$ range of 0.5 to 20 hours, and random error (CV = 15%) at each simulated concentration.

significant underestimate of AUC_{∞}. Therefore, compounds with ($C_{6h}/C_{max} \times 100\%$) > 80% should be flagged with an indication that a significant contribution to AUC_{∞} occurs after the 6-hour time point.

Is a Single-time Point Concentration Viable for Oral Screening?

An empirical exercise was done with the 100-compound test set, to assess whether data from a single time point after PO dosing was correlated to the overall 6-hour exposure (AUC_{6h}); the 3-hour plasma concentration (C_{3h}) had the best correlation (r^2 = 0.95, slope = 5.4, Figure 4A). The simulated data also demonstrate this relationship between C_{3h} and AUC_{6h} (r^2 = 0.95, slope = 5.1, Figure 4B) when the typical rat PK parameters are used (MAT of 0.5-5 hours, terminal $t_{1/2}$ range of 0.5-20 hours, 1- or 2-compartment models, and added random variability [15% CV] to the simulated concentration levels). After identification of this relationship with the 100-compound test set, the linear relationship was evaluated with our entire screening database (5298 compounds) and had remarkable predictability (r^2 = 0.96). Thus, these analyses suggest that a single time point collected 3 hours after a PO dose could be used to predict AUC and rank order compounds. Nonetheless, it was decided not to pursue a single-time point screening approach at this time because the productivity gains are much less than 6-fold (despite 6-fold fewer plasma samples) because of the labor involved in dose preparation, animal dosing, and analytical method development. In addition, when there are 6 data points, the concentration-time profiles can be checked for "smoothness" and the ($C_{6h}/C_{max} \times 100\%$) can be used as described above to understand the reliability of the truncated area. It was reported that AUC can be obtained by analyzing 1 pooled sample from all the time points with an appropriate pooling procedure[6]; however, this pooling procedure was not adopted since is not easily automated and it does not provide a concentration-time profile.

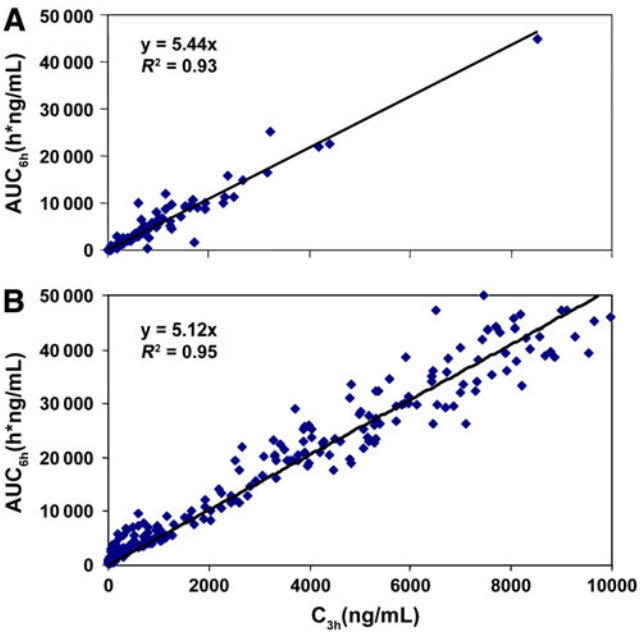

Figure 4. Correlation of AUC_{6h} and C_{3h}: (A) C_{3h} versus AUC_{6h}, from CARRS profiles obtained with 100 compounds; (B) C_{3h} versus AUC_{6h}, from simulated profiles using 1- and 2-compartment models with an MAT range of 0.5 to 5 hours, a terminal $t_{1/2}$ range of 0.5 to 20 hours, and random error (CV = 15%) at each simulated concentration.

What is a "Good" AUC from PO Screening Studies?

F is an important parameter for orally-administered compounds and is more tangible than an AUC value. It is generally accepted that F ≥ 20% is desired for orally administered drugs to minimize interpatient variability and to avoid large clinical doses. F is affected mainly by absorption (F_A) and first-pass hepatic elimination (F_H) and usually is obtained by comparing the AUC_{∞} after IV and oral routes. Since rapid oral screening only provides AUC rather than F, it raises the following questions: (1) how frequently does a high AUC correlate with good bioavailability, (2) what is the maximum F for a given AUC, and (3) where do we "set the bar" for an acceptable screening AUC threshold for a 10-mg/kg dose in rats? These questions were approached in 2 ways: (1) simulations were performed with a variety of

Table 3. Simulated Oral AUC Values at Bioavalibility of 20% With Different F_A and F_H*

F	F_A	F_H	E_H	CL_H (mL/min/kg)	AUC (h*ng/mL)
0.2	1	0.2	0.80	52.0	641
0.2	0.9	0.22	0.78	50.6	733
0.2	0.8	0.25	0.75	48.8	855
0.2	0.7	0.29	0.71	46.4	1026
0.2	0.6	0.33	0.67	43.3	1282
0.2	0.5	0.40	0.60	39.0	1709
0.2	0.4	0.50	0.50	32.5	2564
0.2	0.3	0.67	0.33	21.7	5128
0.2	0.25	0.80	0.20	13.0	10256
0.2	0.202	0.99	0.01	0.6	256410

*F indicates oral bioavailability; F_A, fraction of the dose absorbed into enterocytes that escapes presystemic intestinal elimination; F_H, fraction of compound entering the liver that escapes presystemic intestinal elimination; E_H, liver extraction ratio; CL_H, hepatic clearance; and AUC, area under the concentration curve. The assumptions for the calculations in Table 3 are as follows: (1) compounds are equally distributed between blood cells and plasma (C_{blood}/C_{plasma} = 1), (2) elimination exclusively occurs through liver (CL = CL_H), and (3) F_A and F_H are the major factors that contribute to oral bioavailability ($F = F_A*F_H$). Thus, $AUC_{\infty} = F_A*F_H*Dose/CL_H$, where $F_H = 1 - E_H$, $E_H = CL_H/Q_H$, and Q_H = 65 mL/min/kg in rats.

Table 4. Simulated F Values at AUC of 641 hours*ng/mL With Different F_A and F_H*

AUC (h*ng/mL)	F_A	CL_H (mL/min/kg)	F_H	F
641	1	52	0.20	0.200
641	0.9	51	0.22	0.196
641	0.8	50	0.24	0.190
641	0.7	48	0.26	0.184
641	0.6	46	0.29	0.176
641	0.5	43	0.33	0.167
641	0.4	40	0.38	0.154
641	0.3	35	0.45	0.136
641	0.2	29	0.56	0.111
641	0.1	19	0.71	0.071

*F indicates oral bioavailability; AUC, area under the concentration curve; F_A, fraction of the dose absorbed into enterocytes that escapes presystemic intestinal elimination; F_H, fraction of compound entering the liver that escapes presystemic intestinal elimination; and CL_H, hepatic clearance.

scenarios to understand the interdependency of F and AUC (with random error; CV of 30%) and (2) the relationship between AUC and F was empirically evaluated for these 100 compounds.

If F is fixed at a specific value such as 20%, there are various combinations of F_A and F_H and various AUC values for this condition as shown in Table 3. However, there is a minimum AUC for a given F (641 h*ng/mL at F = 20%), and it corresponds to the case where absorption is complete (F_A = 1) and the bioavailability is limited by the first-pass effect. Similarly, for a given AUC such as 641 h*ng/mL, there are various combinations of F_A and F_H as shown in Table 4. The maximum F for a given AUC is achieved when F_A = 1 and the AUC value is limited by clearance. It is now clear that the maximum F for a given AUC or minimum AUC for a given F occurs when absorption is complete. This evaluation of F and AUC was extended to F values other than 20%, and the 95% CI of the minimum AUC (at F_A = 1) was estimated (Table 5). These numbers represent the relationship between the maximum F for a given AUC or the minimum AUC for a given F. Based on the results in Table 5, AUC screening results at 10 mg/kg can be categorized as low (F < 20%), moderate (20% < F < 50%), or high (F > 50%); thus, the AUC cutoff values of 500 and 2000 h*ng/mL are proposed because they corresponded to the low end of the 95% CI range at these F values. As a primary screen, it is important not to set the bar too high, which could create false negatives. As shown in Figure 5, CARRS only had 3 compounds that had AUC_{6h} < 500 h*ng/mL and F > 20%, supporting the 500-h*ng/mL cutoff value. The CARRS screening results were categorized as low, moderate, or high based on these AUC cutoff values and compared with the actual results from the full-PK studies (IV/PO). As shown in Table 6, using AUC_{6h} to place compounds into low, moderate, and high oral exposure categories is successful, with correct percentages ranging from 77% to 100%. Most of the compounds that are not placed into the right categories are within 25% of the cutoff values.

The Pros and Cons of CARRS

Even though CARRS can provide a fast readout on oral exposure, it does not provide mechanistic information for the causes underlying the low oral exposure. When discovery programs encounter compounds with low oral exposure, a more defined PK study (eg, IV/PO) and/or in vitro

Table 5. Simulated AUC Values and 95% CI with Different F When Absorption Is Complete (F_A = 1)*

F	F_A	F_H	CL_H (mL/min/kg)	AUC (h*ng/mL)	95%CI of AUC (CV% = 30, n = 8) (h*ng/mL)
0.1	1.00	0.10	59	285	222-348
0.2	1.00	0.20	52	641	499-783
0.3	1.00	0.30	46	1099	855-1343
0.4	1.00	0.40	39	1709	1329-2089
0.5	1.00	0.50	33	2564	1994-3134
0.6	1.00	0.60	26	3846	2991-4701
0.7	1.00	0.70	20	5983	4653-7313
0.8	1.00	0.80	13	10 256	7977-12536
0.9	1.00	0.90	7	23 077	17 948-28 206
0.99	1.00	0.99	1	253 846	197 431-310 262

*AUC indicates area under the concentration curve; CI, confidence interval; F, oral bioavailability; F_A, fraction of the dose absorbed into enterocytes that escapes presystemic intestinal elimination; F_H, fraction of compound entering the liver that escapes presystemic intestinal elimination; CL_H, hepatic clearance; and CV, coefficient of variation.

Table 6. Comparison of AUC_{∞} and AUC_{6h} for Categorizing Oral Exposure in Rats at an Oral Dose of 10 mg/kg*

	Full-PK Results Compounds With AUC_{∞}		CARRS Results Compounds With AUC_{6h}		Percentage When AUC_{6h} Places Compounds Into the Correct Category
Exposure Category	AUC (h*ng/mL)	Number of Compounds	AUC (h*ng/mL)	Number of Compounds	
Low	<500	10	<500	10	100
Moderate	500-2000	34	<500	7	
			500-2000	26	76
High	>2000	56	<2000	7	
			>2000	50	89

*AUC indicates area under the concentration curve; full-PK, conventional pharmacokinetic procedure; and CARRS, cassette accelerated rapid rat screen.

absorption, distribution, metabolism, and excretion (ADME) studies such as solubility, permeability, microsomal stability, and plasma stability should be performed to investigate the major cause(s) for low oral exposure. However, most of the time, structural modification aimed to improve one property can be detrimental to other properties. For example, improving permeability might reduce solubility and reduce microsomal stability. Thus, in vivo oral exposure provides a more integrated end point than individual in vitro assays. Obtaining in vivo data directly is more reliable than predicting oral exposure based on combined in vitro properties, especially when transporter systems are involved in absorption or disposition.

Historical Screening Efficiency of CARRS—Is It a Worthwhile Filter?

Based on the empirical and theoretical relationships described above, a lower limit for acceptable AUC (500 h*ng/mL) was proposed since this may represent the lowest possible AUC for a drug with 20% oral bioavailability. Analogously, the lowest possible AUC for a drug with 50% oral bioavailability can be estimated at 2000 h*ng/mL (Table 5), which then defines a "moderate" AUC range between 500 and 2000 h*ng/mL; AUC values higher than 2000 h*ng/mL are thus considered "high." Screening results from the full database (5298 compounds) were placed into these 3 bins to assess the filtering efficiency of CARRS. If most of the compounds evaluated as part of routine discovery screening had AUC values lower than the AUC threshold of 500 h*ng/mL, then the rationale for doing high-throughput oral screening would be strengthened. Indeed, 50% of these compounds had low AUC values (<500 h*ng/mL), 25% had moderate AUC (500 to 2000 h*ng/mL), and 25% had high AUC (>2000 h*ng/mL). Therefore, this high throughput oral screen is an effective primary filter such that the majority of compounds can be removed from further consideration with a single experiment requiring less than 15 mg of drug.

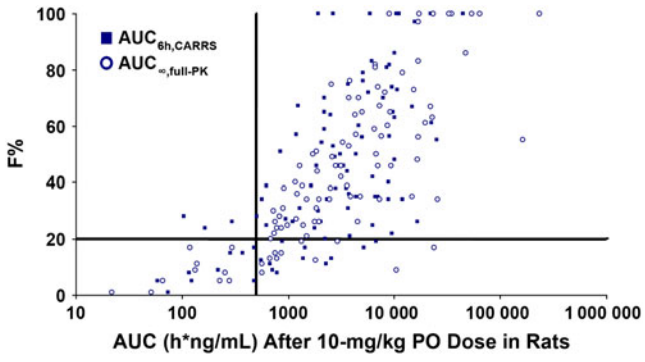

Figure 5. Distribution of compounds relative to the AUC cutoff value of 500 hours*ng/mL and F of 20% with an oral dose of 10 mg/kg in rats.

CONCLUSIONS

The current evaluation indicates that the truncated AUC_{6h} values obtained from CARRS (pooled plasma samples from 2 individually dosed rats with an oral dose of 10 mg/kg) provide oral exposure information that is comparable to the results obtained with more rigorous conventional PK studies for drug discovery compounds. Compounds that have substantial AUC beyond 6 hours can be identified when ($C_{6h}/C_{max} \times 100\%$) values exceed 80%. Based on results from 100 compounds from 15 structurally distinct chemotypes and pharmacokinetic simulations, a "threshold" for acceptable AUC (500 h*ng/mL) was established for a 10-mg/kg oral screening dose in rats. While, in practice, many discovery programs adopt a higher (more stringent) threshold, compounds below this level of exposure can be discarded with confidence and are very likely to be poor candidates for development. With the AUC threshold set at 500 h*ng/mL, 50% of the 5298 compounds screened in the last 2 years

at Schering-Plough have been discarded, which, in our view, has provided a useful decision gate to avoid wasting discovery resources on nonviable candidates.

ACKNOWLEDGMENTS

The authors would like to thank the following people for their contributions in CARRS and full-PK studies, which allowed this evaluation to be possible: Kathleen Amatulli, Jean-Marc Brisson, Lisa Broske, Jennifer Frick, Yunsheng Hsieh, Cymbelene Nardo, Adrienne Pena, Christine Smith, Gregory Tucker, Samuel Wainhaus, Ganfeng Wang, Shiyong Wang, Xiaoying Xu, and Qiao Zhou.

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