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Integrated oral bioavailability projection using in vitro screening data as a selection tool in drug discovery

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Abstract

The objective of the analysis described herein is to examine the in vitro/in vivo relationship of estimated bioavailability values and also the applicability of the estimated in vitro bioavailability to lead candidate selection in drug discovery. To this end, in vitro ADME data from screening assays as well as in vivo rat pharmacokinetic (PK) data were compiled for 140 compounds across therapeutic areas from the Pfizer library in Ann Arbor. The compounds span a broad range of structural types, including neutral, basic, and acidic compounds. Solubility and Caco-2 permeability data from in vitro ADME screening were used to calculate the fraction dose absorbed (FDp) using the physiologically based IDEA® model. In vitro metabolic stability ($t_{1/2}$) from human and rat liver microsomal incubations was converted to an in vitro intrinsic clearance value (CL'_{int}), which was then scaled up to reflect in vivo clearance (CL) and hepatic extraction as described by Obach et al. [J. Pharmcol. Exp. Ther. 283 (1997) 46]. Subsequently, the in vitro/in vivo relationship between the measured bioavailability (F_{obs}) in rats and the estimated bioavailability (F_{est}) from FDp and predicted CL values was examined. The observed data suggest that compounds with low estimated in vitro bioavailability ($F_{est} < 15\%$) are more likely to have low in vivo bioavailability ($F_{obs} < 30\%$). Therefore, the present study indicates that in vitro estimation of bioavailability is an efficient tool to eliminate compounds having low bioavailability prior to in vivo characterization and therefore can be used to reduce attrition due to poor ADME properties in drug development.

Keywords: IDEA model; Bioavailability; In vitro prediction; Drug discovery

1. Introduction

A major challenge to pharmaceutical scientists in drug discovery is in the optimization and selection of lead compounds from the abundance of new chemical entities (NCEs) in early drug discovery with the best chances for success. Many studies have supported that

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poor pharmacokinetic properties heavily contribute to compound failure or at a minimum, difficulty of the compound to progress through drug development (Prentis et al., 1998; Eddershaw et al., 2000). Therefore, to reduce attrition in drug development, it is important to identify the major pharmacokinetic hurdles of drug candidates and assess the developability of a compound early in drug discovery. Recently, in vitro ADME screening assays have been established to generate significant amounts of in vitro ADME data (e.g. solubility, permeability, metabolic stability, drug–drug interaction potential, etc.) in a high throughput format early in drug discovery. The availability of such in vitro ADME data enables their use to characterize and predict in vivo pharmacokinetics of drugs (Venkatesh and Lipper, 2000; Hodgson, 2001). Consequently, there is growing interest to establish the in vitro/in vivo correlation of pharmacokinetic parameters in order to select lead candidates having favorable pharmacokinetic properties without any significant in vivo evaluation.

Many studies have been published exploring different approaches to establish in vitro/in vivo correlations by combining metabolic clearance and fraction dose absorbed to ultimately project human pharmacokinetic parameters (Obach et al., 1997; Obach, 1999; Shibata et al., 2000; Lave et al., 1997; Naritomi et al., 2001; Chiou and Barve, 1998; Chiou et al., 2000). However, there have been limited attempts to integrate multiple in vitro ADME parameters from high throughput screening to estimate bioavailability of NCEs and subsequently to use the estimated in vitro bioavailability as a screening filter in the early stages of drug discovery (Theil et al., 2003; Parrot and Lavé, 2002; Bohets et al., 2001; Waterbeemd et al., 2001; Waterbeemd, 2001). Since bioavailability is affected by multiple factors, including solubility, permeability, and first pass metabolism, in vitro biopharmaceutical/pharmacokinetic parameters from screening assays should not be used in isolation to project oral bioavailability unless the critical factor limiting bioavailability has been clearly identified for the particular compound or chemical series. Therefore, if multiple in vitro screening measurements can be integrated to adequately estimate bioavailability, it will greatly reduce the cost and time consuming in vivo pharmacokinetic evaluation of discovery compounds. In order for an integrated model to gain acceptance by discovery project teams (relative to discrete models), it must clearly demonstrate the ability to choose a greater proportion of ‘good’ compounds with a minimized risk for eliminating otherwise ‘good’ compounds from the discovery project. The latter compounds are termed as ‘false negatives’ and are a general cause for concern in early discovery. Additionally, such an integrated model should be broadly applicable to early discovery programs across structural series and therapeutic areas.

The present study examines the in vitro/in vivo relationship of estimated bioavailability values and also the applicability of the predicted bioavailability to the lead candidate selection in early drug discovery. The methodology used in this study was to combine in vitro data with a commercially available software package (IDEA®, LION Bioscience Inc., San Diego, CA, USA) with the goal of implementing this tool in early drug discovery. In vitro ADME data from screening assays as well as in vivo rat PK data were compiled for 140 compounds across a broad range of structural chemotypes from the Pfizer Chemical library in Ann Arbor. A retrospective analysis of in vitro/in vivo relationship was performed between the measured bioavailability in rats and the in vitro bioavailability estimated from integration of in vitro screening data.

2. Methods

2.1. Fraction dose absorbed (FDp)

Fraction dose absorbed in portal vein (FDp) was estimated by using the IDEA® model (LION Bioscience Inc., 92121) (Theil et al., 2003; Parrot and Lavé, 2002). As shown in Fig. 1, the input factors used in IDEA® model to estimate FDp values are solubility at various pH values (1.5, 5.0, 6.5, 7.0, and 7.5), Caco-2 permeability ($P_{app(A\rightarrow B)}$) measured at 20 μM, log P and the efflux ratio ($P_{app(B\rightarrow A)}/P_{app(A\rightarrow B)}$). Experimental procedures to determine Caco-2 permeability, solubility, and log P were discussed previously (Stilgenbauer et al., 2000a,b; Kibbey et al., 2001). Solubility at various pH values was calculated based on measured solubility at pH 6.5 and pK_a from in vitro ADME screening as described by Flynn et al. (Kramer and Flynn, 1972; Horter and Dressman, 1997).

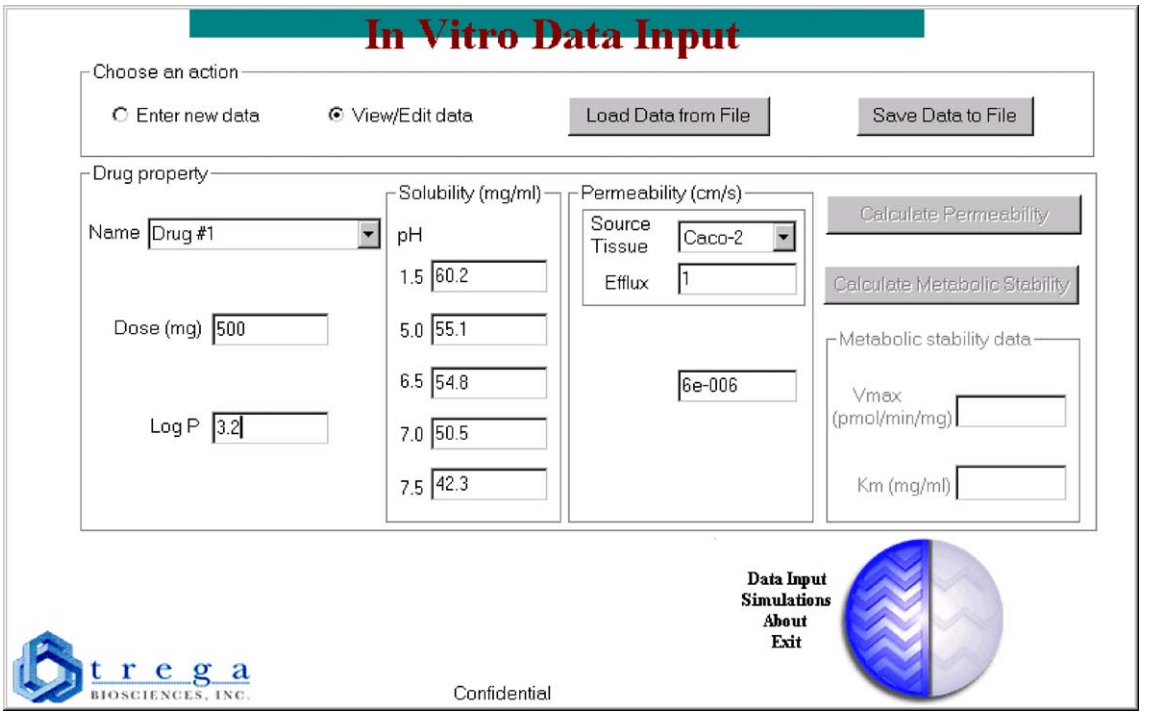

Fig. 1. In vitro data input to an IDEA® model.

2.2. Clearance (CL)

In vivo clearance was projected based on in vitro disappearance half-life measured from rat and human liver microsomal incubation by using the following equations, as summarized by Obach et al. in their previous report (Obach, 1999).

$$\mathrm{CL}'_{\mathrm{int}} = 0.693 \times \frac{1}{t_{1/2}\,(\mathrm{min})} \times \frac{\mathrm{gm\ liver\ wt}}{\mathrm{kg\ body\ wt}} \times \frac{\mathrm{ml\ incubation}}{\mathrm{mg\ microsomal\ prot}} \times \frac{45\ \mathrm{mg\ microsomal\ prt}}{\mathrm{gm\ liver\ wt}} \quad (\mathrm{CL}'_{\mathrm{int}}:\ \text{hepatic intrinsic clearance}) \tag{1}$$

$$\mathrm{CL} = \frac{Q \times \mathrm{CL}'_{\mathrm{int}}}{Q + \mathrm{CL}'_{\mathrm{int}}} \quad (Q:\ \text{hepatic blood flow}) \tag{2}$$

The use of in vitro hepatic microsomal intrinsic CL data to predict systemic CL was based on several assumptions: (1) metabolic CL is the primary CL mechanism of compounds, (2) the liver is the major CL organ, (3) oxidative microsomal metabolism is the predominant route of metabolism (compared with non-microsomal metabolism and conjugative metabolism), and (4) metabolic rates and enzyme activities measured in vitro are truly reflective of those that occur in intact systems in vivo.

Experimental procedures to determine in vitro disappearance half-life in microsomes were discussed previously (Stilgenbauer et al., 2000a,b).

2.3. Bioavailability (F, %)

Oral bioavailability can be determined using the following equations.

$$F = F_{\mathrm{a}}(1 - E_{\mathrm{h}}) = F_{\mathrm{a}}\left(1 - \frac{\mathrm{CL_h}}{Q}\right) \tag{3}$$

where F_{a} is fraction dose absorbed; E_{h} is hepatic extraction ratio; $\mathrm{CL_h}$ is hepatic blood clearance, and Q is hepatic blood flow (Rowland and Tozer, 1995).

Assuming that hepatic metabolism is the primary clearance mechanism of compounds, $\mathrm{CL_h}$ = CL

where CL is systemic clearance, thus

$$F = F_a\left(1 - \frac{CL}{Q}\right) \quad (4)$$

In the present study, in vitro projected oral bioavailability (F_{est}) was estimated based on FD_p (projected F_a by using computational IDEA model: see details in Section 2) and projected in vivo clearance (CL) (see Eqs. (1) and (2)). Therefore,

$$F_{est} = FD_p\left(1 - \frac{CL}{Q}\right) \quad (5)$$

where $Q = 70$ ml/min/kg in rats and 21 ml/min/kg in humans (Altman and Dittmer, 1972).

2.4. Statistical analysis

For each method, a test was performed to assess the association between each method's classification and the in vivo classification. The null hypothesis for each test was no association and the continuity-adjusted c^2 statistic (c^2_{adj}) was used as the statistic for the test.

3. Results

3.1. In vitro profiles of compounds

In vitro profiles of structurally diverse 140 compounds are illustrated in Fig. 2. Binning which is a classification scheme to categorize numerical data into various segments (e.g. high, medium, and low) allows for a simple assessment of the property's distribution and also to determine where a potential liability exists. For example, it is clear that the majority of the compounds have poor aqueous solubility (86/140) or poor Caco-2 permeability (65/140) suggesting that poor absorption is likely to be a contributing factor for poor in vivo performance for these compounds. The choice of 10 μg/ml as a minimum acceptable solubility is based on Lipinski's analysis where for a compound with high permeability and a dose of 1 mg/kg, aqueous solubility must be equal or greater than 10 μg/ml for complete absorption in humans (Lipinski, 2000). The low permeability cut-off was established based on in-house data for the Biopharmaceutical Classification Scheme (BCS) compounds in our Caco-2 assay.

3.2. Classification of drugs by using in vitro and in vivo bioavailability

Since the methodology applied to predict in vivo clearance does not include non-microsomal routes of elimination nor non-metabolic elimination pathway (see Section 2), a linear relationship between in vitro and in vivo estimates of bioavailability would not be anticipated. Indeed, there is no linear correlation between in vitro and in vivo estimates of bioavailability (discussed in details later). However, the observed data suggest that compounds with low estimated in vitro bioavailability (F_{est}) are more likely to have low in vivo bioavailability in rats ($F_{obs} < 30\%$). Therefore, another aspect of the in vitro/in vivo relationship was examined based on *drug classification* by using both F_{est} and F_{obs}.

3.2.1. Set the optimal boundary for the classification of compounds

It is well recognized that compounds with low bioavailability tend to have higher clinical variability as demonstrated by Hellriegel et al. (1996). Based on the report from Hellriegel et al. in conjunction with our internal experience, in vivo bioavailability of 30% was used as the minimum threshold of in vivo bioavailability (F_{obs}) to assess the developability of compounds. To find an optimum range of F_{est} (%) for classifying low orally available compounds ($F_{obs} < 30\%$), misclassification rates were examined as shown in Fig. 3. The ideal region for classifying compounds would simultaneously minimize both false negative and false positive rates. Fig. 3 suggests that classifying observations with F_{est} less than 15% as low bioavailability provides an optimal balance between both false negative and false positive rates.

3.2.2. Binning relationship between F_{obs} and F_{est}

As shown in Fig. 4, there is good correlation between in vitro estimates of bioavailability (F_{est}) for rats and humans. However, 12 compounds out of 140 compounds were classified differently by using in vitro estimates of bioavailability (F_{est}) in humans or rats. For example, some of compounds (open circles in Fig. 4) showed greater than 15% of F_{est} in rats but less than 15% of F_{est} in humans. Therefore, to minimize false negatives, greater than 15% of F_{est} in either

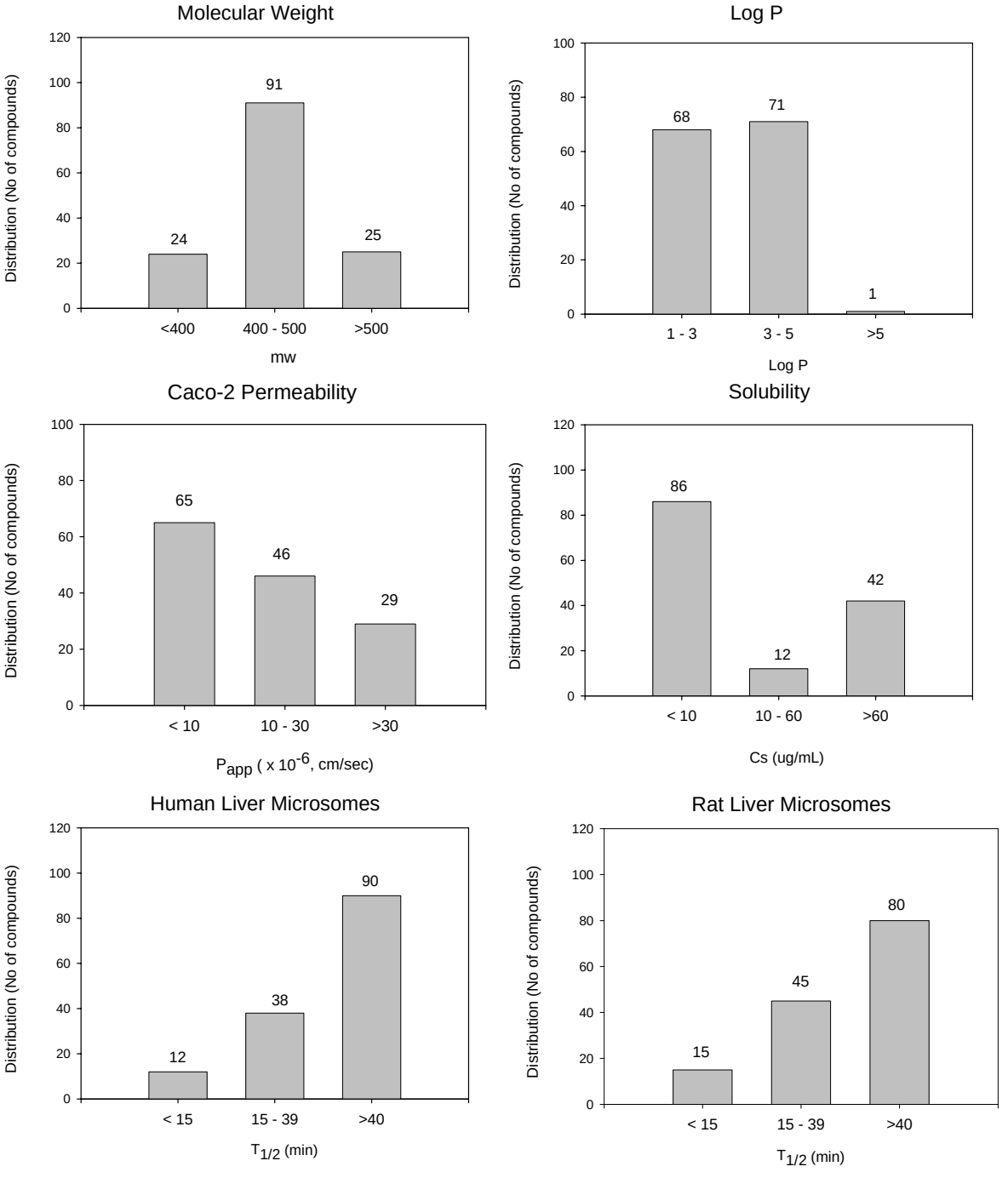

Fig. 2. Distribution of compounds properties in the data set: human liver microsomal $t_{1/2}$ (min) < 15, 15–39, and ≥40 is equivalent to $CL_{int}{}' > 87$, $CL'_{int} = 34$–87, and $CL'_{int} < 34$ ml/min/kg, respectively. Rat liver microsomal $t_{1/2}$ (min) < 15, 15–39, and ≥40 is equivalent to $CL'_{int} > 166$, $CL'_{int} = 64$–166, and $CL'_{int} < 64$ ml/min/kg, respectively.

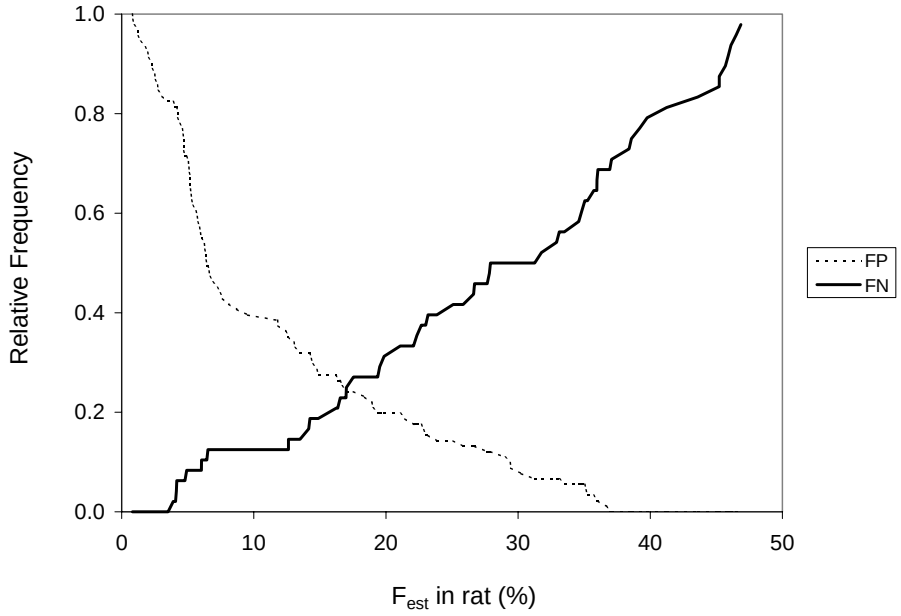

Fig. 3. In vitro estimated bioavailability in rat versus relative frequency of false negative and false positive (misclassification rates): FP: false positive, FN: false negative.

rat or humans was used for the compound selection to achieve greater than 30% bioavailability in vivo. As illustrated in Fig. 5, among 140 compounds, 55 compounds were classified as the Category I (less than 15% of F_{est} and less than 30% of F_{obs}), 9 compounds into Category II (false negative), 47 compounds into Category III (greater than 15% of F_{est} and greater than 30% of F_{obs}), and 29 compounds into Category IV (false positive).

Collectively, 73% of compounds were classified into the correct category using this approach without significant false negatives (6%). Statistical analysis

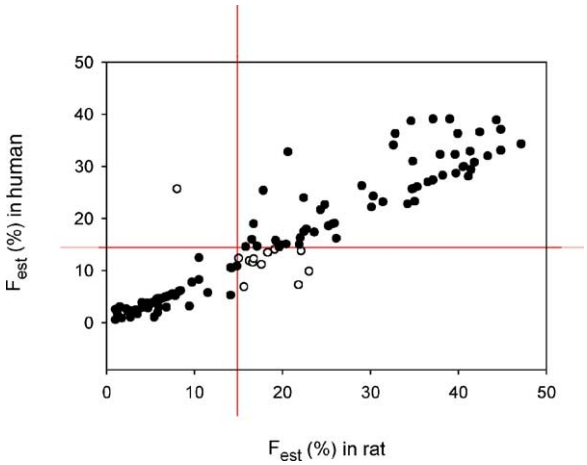

Fig. 4. In vitro estimated bioavailability in humans vs. in vitro estimated bioavailability in rats: open circles represent the compounds that were classified differently in humans and rats.

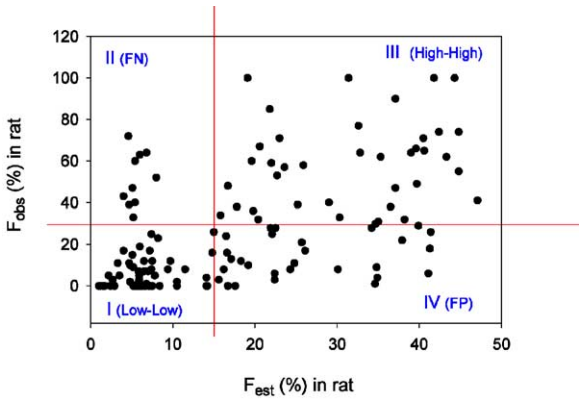

Fig. 5. Binning relationship between in vitro/in vivo bioavailablity. Each category represents as follows; Category I: less than 15% of F_{est} and less than 30% of F_{obs}, Category II: FN, false negative, Category III: greater than 15% of F_{est} and greater than 30% of F_{obs}, and Category IV: FP, false positive.

Table 1
Comparison between discrete analysis (single parameter analysis) and integrated in vitro bioavailability projection method for the classification of compounds

In vitro	In vivo (Rat)	
	Bad (F_{obs} < 30%)	Good (F_{obs} > 30%)
(A) Integrated in vitro bioavailability projection method		
Bad (F_{est} < 15%)	55 (39.3%)	9 (6.4%)
Good (F_{est} > 15%)	29 (20.7%)	47 (33.6%)
(B) Discrete analysis		
Bad (C_s < 10 or RLM < 15 or P_{app} < 10)	73 (52.1%)	24 (17.1%)
Good (C_s > 10, RLM > 15 and P_{app} > 10)	11 (7.9%)	32 (22.9%)

C_S = solubility (μg/ml), RLM = $t_{1/2}$ in rat microsome (min) and P_{app} = Caco-2 permeability ($\times 10^{-6}$, cm/s). RLM < 15 is equivalent to CL'_{int} > 166 ml/min/kg.

(adjusted chi-square test) indicated that there is a significant association (P-value <0.0001) between this in vitro and in vivo classification method. Furthermore, the results were compared with the discrete analysis that utilizes each in vitro parameter separately to classify compounds. As summarized in Table 1, the discrete analysis classified 75% of compounds into the correct category but it generated a threefold higher rate of false negatives (17%) than the integrated in vitro projection method (6%). Additionally, the integrated model discriminated 'good' compounds from the 'bad' compounds at a higher rate than the discrete model (22.9% discrete versus 33.6% integrated models). Thus, the observed data suggest that integrated in vitro projection of bioavailability (F_{est}) can be an efficient tool to screen out compounds having low bioavailability prior to in vivo characterization in early discovery.

4. Discussion

Early assessment of ADME profiles of compounds is important in reducing attrition in drug discovery, and consequently expediting the drug development process. The major pharmacokinetic parameters taken into account at lead candidate selection stages are systemic exposure (AUC), bioavailability (F), and plasma half-life ($t_{1/2}$). Systemic exposure (AUC) is assessed to determine whether systemic exposure associated with efficacy can be achieved with clinically applicable doses, and thus the systemic exposure should be considered in the conjunction of potency/efficacy of the drugs. Since the desired systemic exposure of drug candidates depends on the pharmacological activity against targets, the first cut-off of AUC as selection criteria of lead candidates will be target specific or project specific. Plasma half-life is also an important factor to support twice a day dosing (b.i.d.) or once a day dosing (q.d.) regimen and it is altered by the change of other pharmacokinetic parameters, such as clearance and volume of distribution. In vitro ADME screening data provide useful information regarding pharmacokinetic parameters deemed to be important in early discovery. Therefore, for the optimization of plasma half-life, there have been many different approaches to predict those factors responsible for affecting in vivo plasma half-life from in vitro ADME data (Obach et al., 1997; Obach, 1999; Shibata et al., 2000; Lave et al., 1997; Naritomi et al., 2001; Chiou and Barve, 1998; Chiou et al., 2000; Poulin and Theil, 2002).

Oral bioavailability is a particularly important selection criterion for lead candidates in early drug discovery, considering that oral administration is the most desirable route of administration. Therefore, there have been different approaches to predict bioavailability (ultimately for humans) (Theil et al., 2003; Parrot and Lavé, 2002; Bohets et al., 2001; Waterbeemd et al., 2001; Waterbeemd, 2001) but still it is needed to further refine those approaches for more accurate prediction of human bioavailability. In order to meet the throughput demand of discovery, and to identify lead compounds in a timely manner, a practically facile approach is more appropriate to predict bioavailability of compounds in early drug discovery while a more refined approach is required for compounds in advanced stages of drug development. Therefore, the present study is focused on the utility of in vitro screening data in predicting bioavailability of early discovery compounds. As shown in Fig. 2, binning 1 property allows for a simple assessment of the compound's property distribution and to determine where a potential cause of low bioavailability exists. However, since bioavailability is determined by combining multiple factors including solubility, permeability and metabolic stability, in vitro ADME data from screening assays should not be used in isolation to project oral bioavailabil-

ity unless the critical factor limiting bioavailability has been clearly identified for particular compounds or chemical series. Indeed, the discrete analysis that utilizes each in vitro ADME data separately produced a significant rate of false negatives (17%) compared to the integrated model (6%) further highlighting this caveat (Table 1).

A set of 140 structurally diverse compounds was selected across therapeutic areas, and the relationship between in vitro and in vivo estimates of bioavailability was examined. The observed data suggest that compounds with low estimated in vitro bioavailability (F_{est}) are more likely to have low in vivo bioavailability ($F_{obs} < 30\%$). As shown in Fig. 5, using 0–15% of F_{est} as an indicator of compounds with low in vivo bioavailability ($F_{obs} < 30\%$) could classify 73% of the compounds into the correct category, with 6% classified as false negatives and 21% classified as false positives. On the other hand, although the discrete analysis classified 75% of compounds into correct category it chose, only 22.9% of compounds that ultimately demonstrated adequate in vivo bioavailability (>30%) compared to 33.6% by the integrated model. While the integrated method does not offer a superiority when solely considering elimination as the goal (40% versus 52%), the combination of higher true positives and low false negatives of the integrated model supports the integrated method as a complementary tool to the discrete analysis for practitioners of early ADME screening in support of various drug discovery programs.

As with any model, our analysis of this integrated approach yields both false positives and false negatives. The observation of false positives can be explained in several ways. First, the present approach utilizes in vitro hepatic microsomal intrinsic CL data to predict systemic CL. Therefore, if the compounds do not meet the assumptions described in Section 2, errors in both CL and bioavailability projections will occur. For example, if Phase II reactions (conjugation) are the major elimination route for the compounds, the present approach using microsomal stability data will underestimate hepatic clearance of those compounds and subsequently in vitro estimates of bioavailability (F_{est}) will be greater than in vivo bioavailability (false positive). Second, the IDEA® model does not incorporate a GI stability factor into the calculation of fraction dose absorbed, and thus if compounds undergo significantly intestinal metabolism, or chemical instability in gastric acid, then the bioavailability of those compounds will be overestimated by the present approach using calculated FD_p values from IDEA® model.

While false positive data are of less concern in the present approach, false negative data should be more carefully evaluated in order to reduce the chance of eliminating promising compounds. In the present study, only 6% of tested compounds were classified as the false negatives. The specific reasons for these failures were not examined further in this study. However, based on evidence from literature and our experience, several reasons could contribute to these observations. First, if active transport mechanism is involved in the intestinal drug absorption, Caco-2 data may underestimate the intestinal absorption of those compounds due to lower expression of active uptake transporters relative to in vivo expression, and subsequently underestimate the fraction dose absorbed. Consequently, in vitro estimates of bioavailability using underestimated fraction dose absorption should be less than measured values in vivo. Second, if there is significant difference between in vitro and in vivo solubility (e.g. increased solubilization by bile salts in vivo, etc.), the IDEA® model may underestimate the fraction dose absorption of poorly soluble drugs and subsequently underestimate the in vivo bioavailability. Third, if the metabolic rates and enzyme activities measured in vitro are significantly different from those that occur in vivo, the present approach using in vitro metabolic stability data may overestimate the in vivo clearance and subsequently may result in the underestimation of in vivo bioavailability.

While there is a need for further clarification of false negatives, this does not diminish the utility of our approach for the following reasons: (a) the risk was superior to the risk associated with decision making based on discrete variables (6% versus 17% false negative); (b) there was no discernible trend in the false negatives based on the scaffold or template suggesting a systematic deviation; and (c) the risk associated with 6% of false negative is generally deemed as acceptable at the early stages in drug discovery.

In conclusion, the results suggest that the present approach using in vitro estimate of bioavailability is useful (1) to reduce the time and cost of in vivo animal studies and (2) eliminate compounds having low bioavailability prior to in vivo characterization. This

study also underscores the need to integrate various in vitro ADME data in a scientifically sound and practically facile manner in order to meet the throughput and data turnaround times in early drug discovery.

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