ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Strategic Use of Plasma and Microsome Binding To Exploit in Vitro Clearance in Early Drug Discovery

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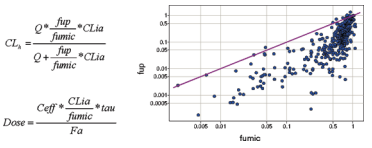
ABSTRACT Apparent intrinsic clearance (CLia) determined from microsomal stability assays is a cornerstone in drug discovery. Categorical bins are routinely applied to this end point to facilitate analysis. However, such bins ignore the interdependent nature of apparent intrinsic microsome clearance on several ADME parameters. Considering CLia as a determinant for both metabolic stability and potential dose is more appropriate. In this context with proper accounting for nonspecific binding to microsomes and plasma, consideration of compounds with higher CLia may be warranted. The underlying benefit is the potential increase in the number of hits or chemical diversity for evaluation during the early stages of programs.

KEYWORDS Plasma binding, microsome binding, fraction unbound, clearance, dose, ADME parameters



For successful drug discovery, multiple ADME end points need to be considered, monitored, and optimized simultaneously. As a consequence, categorical bin values are routinely set for these end points to facilitate stoplight type analysis.[1] However, setting these bin values without considering the *interdependent* nature of these end points may be inappropriate. This study will show the importance and strategic benefits of using both the stage of a project and an integrated parameter such as dose to guide appropriate clearance ranges or bins, rather than the bin values for the human microsome stability assay alone.

In assessing human in vivo metabolic stability, in vitro systems are routinely used,[2] and high-throughput screening of compounds in human liver microsomes has become commonplace.[3,4] In these in vitro systems, metabolic stability is reported as either apparent microsomal intrinsic clearance (CLia)[1] or half-life ($T_{1/2}$).[5] Using the well-stirred model (eq 1) and assuming that the fup/fumic ratio is 1, categorical ranges as described in eqs 2 and 3 translate to human hepatic extraction ratios [Er = CL_h/hepatic blood flow (Q = 20 mL/min/kg)] of 30 and 70%, respectively (cytochrome P450 concentration = 0.25 μM).

$$CL_h = \frac{Q \times \text{fup} \times \frac{\text{CLia}}{\text{fumic}}}{Q + \text{fup} \times \frac{\text{CLia}}{\text{fumic}}} = \frac{Q \times \frac{\text{fup}}{\text{fumic}} \times \text{CLia}}{Q + \frac{\text{fup}}{\text{fumic}} \times \text{CLia}} \quad (1)$$

$$\text{low CLia} < 8.7 \text{ mL/min/kg} < \text{moderate CLia} < 46 \text{ mL/min/kg} < \text{high CLia} \quad (2)$$

$$\text{long } T_{1/2} > 112 \text{ min} > \text{moderate } T_{1/2} > 21 \text{ min} > \text{short } T_{1/2} \quad (3)$$

As a consequence, for the ranges shown in eqs 2 and 3, project teams usually avoid considering compounds with CLia > 46 mL/min/kg or $T_{1/2}$ < 21 min to avoid a high hepatic extraction (Er > 70%).

In the prediction of hepatic clearance (CL_h) from microsomes, nonspecific, noncovalent binding of compounds to plasma (fup) and microsomes (fumic) needs to be considered.[6] However, assuming that the fup/fumic ratio is 1 usually remains an untested assumption until much later in a project. More critically is that the existence of this assumption is frequently forgotten and the impact of this assumption goes unnoticed. The impact of this assumption could be negligible if changes in binding to plasma and microsomes relative to lipophilicity are proportional.

To investigate the validity of this assumption, fup and fumic values for 416 compounds in the Pfizer database with disparate structure and physicochemical properties were measured and analyzed. As shown in Figure 1, the fup and fumic values for the majority of the compounds are dissimilar. Most of the compounds are below the unity line, indicating that the fup/fumic ratio is <1. Hence, assuming a fup/fumic ratio of 1 and that a proportional change in binding to plasma and microsome relative to lipophilicity is occurring does not appear to be supported by data (Figure 1).

Of more significance, 70% of the compounds have fup/fumic ratios ≤0.4 (Figure 2). The theoretical consequence of this subunity ratio on the well-stirred model for calculating CL_h is illustrated in Table 1.

Received Date: December 8, 2009
Accepted Date: January 24, 2010
Published on Web Date: February 03, 2010

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DOI: 10.1021/ml900012h | *ACS Med. Chem. Lett.* **2010**, *1*, 50–53

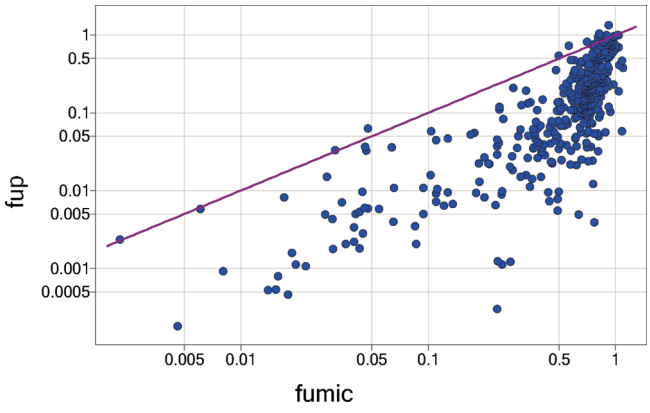

Figure 1. Comparison of microsome binding (fumic) and plasma binding (fup) for 416 compounds in the Pfizer database. Most of the compounds are below the unity line, indicating the fup/fumic ratio is < 1.

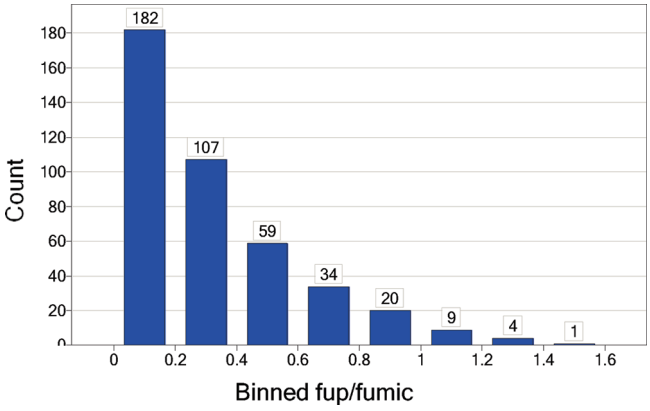

Figure 2. Distribution of fup/fumic ratios. The value above each bar represents the number of compounds in the fup/fumic ratio range. For this data set of 416 compounds, 70% have fup/fumic ≤ 0.4.

As mentioned earlier, with the well-stirred model (eq 1), a CLia of 46 mL/min/kg results in a calculated Er of 70% when fup/fumic is assumed to be 1 (entry 2). However, as a consequence of dissimilar fup and fumic values, higher CLia values yield the same calculated 70% extraction ratio when the fup/fumic ratio is decreased (entries 3−6). While a very high CLia can still yield an acceptable calculated hepatic clearance and extraction ratio when the fup/fumic ratio is very low (entry 6), the same CLia translates to hepatic clearance approaching human hepatic blood flow and complete hepatic extraction if fup/fumic is assumed to be 1 (entry 7). This table indicates that avoiding chemistry space with CLia values >46 mL/min/kg in early stages of drug discovery is based on an erroneous assumption. Compounds with higher CLia values may be considered without suffering an increase in extraction ratio when appropriate fup/fumic ratios are taken into consideration. The underlying drive for considering compounds with higher CLia is to increase the number of hits or chemical diversity for evaluation. A survey of the Pfizer database of microsomal clearances for the past 4 years indicates that increasing CLia from 46 to 150 mL/min/kg could potentially increase the number of compounds for consideration by 60%. Furthermore, a survey of commercial drugs finds several with reported CLia >50 mL/min/kg including top-selling diclofenac (CLia = 189 mL/min/kg, fup = 0.005, and fumic = 1).[6] We stress that the intent of this letter is to highlight and allow the expansion of chemistry space early on in a project by appropriately considering the fup/fumic ratio. Although commercial drugs with high CLia values exist, for the majority of drugs, it is anticipated that clearance will be lower as simultaneous optimization of potency and clearance is a necessity for success and part of the natural progression of projects.

Table 1. Theoretical Calculation of Human CL_h and Er from CLia with Varying fup/fumic Ratios Using the Well-Stirred Model (Eq 1)[a]

entry	$T_{1/2}$ (min)	CLia (mL/min/kg)	fup/fumic	CL_h (mL/min/kg)	Er[b]
1	112	9	1.0	6	0.30
2	21	46	1.0	14	0.70
3	8	118	0.4	14	0.70
4	6	156	0.3	14	0.70
5	4	236	0.2	14	0.70
6	2	463	0.1	14	0.70
7	2	463	1.0	19	0.96

[a] Increasing CLia yields the same calculated hepatic clearance and extraction ratio when the fup/fumic ratio is proportionally decreased. [b] Er = CL_h/Q, where Q = human hepatic blood flow of 20 mL/min/kg.

The balance for considering higher metabolically cleared compounds is, however, reflected in a likely increase in the apparent dose of the compound. The required low fup/fumic ratios can be achieved with varying levels of fumic. However, the impact of a low fumic relative to a high fumic is significant on the eventual apparent dose of the compound.

At steady state, dose is a function of potency or effective concentration (Ceff) and intrinsic clearance (CLi) as well as dosing interval (τ) and fraction absorbed (Fa) (eq 4). If oxidative clearance is the major mechanism of clearance, then CLi reflects intrinsic microsomal clearance. If higher microsomal clearance is considered, then there must be a corresponding decrease in Ceff or an increase in potency, if no offset increase in the final dose can be tolerated.

$$\text{dose} = \frac{\text{Ceff} \times \text{CLia} \times \tau}{\text{Fa}} \quad (4)$$

Because intrinsic microsomal clearance (CLi) is determined by dividing the apparent microsomal intrinsic clearance (CLia) by microsome binding (fumic), eq 4 can be expanded to eq 5.

$$\text{dose} = \frac{\text{Ceff} \times \dfrac{\text{CLia}}{\text{fumic}} \times \tau}{\text{Fa}} \quad (5)$$

The significance of eq 5 is that with increasing CLia, the requisite decrease in Ceff is proportional when fumic = 1 and suprapropotional when fumic ≪ 1 (high binding to microsome), assuming no offset increase in final dose.

Shown in Table 2 are theoretical calculations of doses with changes in Ceff, CLia, and fumic. In these hypothetical

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DOI: 10.1021/ml900012h | ACS Med. Chem. Lett. 2010, 1, 50–53

Table 2. Theoretical Calculation of Doses (Eq 5) with Increasing CLia, Decreasing Ceff, fup/fumic Ratio = 1 or 0.1, and No Change in Fraction Absorbed or Dosing Interval[a]

entry	Ceff (ng/mL)	CLia (mL/min/kg)	fold increase in CLia over entry 7	fumic	dose[b,C] (mg)	fold increase in dose over entry 7
7	100	46		1.0	464	
8	100	118	2.5	1.0	1189	2.5
9	100	118	2.5	0.1	11894	25.5
10	40	118	2.5	1.0	476	1.0
11	4	118	2.5	0.1	476	1.0

[a] The increase in dose is proportional to the increase in CLia when fumic = 1 and greater than proportional when fumic = 0.1. [b] Assuming Fa = 1, QD dosing for a 70 kg person. [c] The dose calculation for entry 9 is shown as an example. $\frac{100\ \text{ng}}{\text{mL}} \times \frac{118\ \text{mL}}{\text{minkg}} \times \frac{1}{0.1} \times \frac{1440\ \text{min}}{\text{day}} \times \frac{1\ \text{mg}}{1000000\ \text{ng}} \times 70\ \text{kg} = 11894$ mg total dose for a 70 kg person per day

examples, during early stages of programs when Ceff has not been optimized (e.g., Ceff = 100 ng/mL) and assuming Fa = 1 and QD dosing for a 70 kg person, a dose of 464 mg is calculated for a compound with a CLia = 46 mL/min/kg (entry 7). When considering a higher CLia, although there is a potential for not increasing Er values (Table 1), there is a calculated increase in dose assuming no change in potency (Ceff). However, the increase in dose is *proportional* when fumic = 1 (entry 8) and *supraproportional* when fumic = 0.1 (entry 9). The increase in dose due to higher CLia can be compensated by an increase in potency (e.g., Ceff = 40 or 4 ng/mL). Again, the compensation in potency is *proportional* when fumic = 1 (entry 10) and *supraproportional* when fumic = 0.1 (entry 11).

The current proposed strategy dissuades the use of hard clearance bin values during early discovery to discard seemingly high clearance compounds on the basis of an assumption but rather to also consider the fup/fumic ratio. This information should then be used in combination with CLia and potency through the use of the dose equation to determine what is unfavorable chemistry space in terms of clearance. Subsequently, compounds with higher CLia values (>46 mL/min/kg) could be evaluated and considered for advancement. Key to the success of this strategy is to use this in early discovery. Any offset in dose as a function of considering higher clearance compounds could potentially be ameliorated by increases in potency. At early stages of a program, potency usually still needs 10× to 100× improvement, while greater chemistry space is made available for consideration and evaluation. It is clear that this required potency increase cannot be achieved by increasing lipophilicity as that would most likely increase clearance and decrease fumic, resulting in a zero-sum gain.[7] Indeed, recent analysis has revealed that successful transformation of screening hits to resulting leads or drug candidates is usually achieved with small changes in lipophilicity,[8] thus further emphasizing the importance in achieving potency increase in the absence of a significant lipophilicity increase. It is also clear that as programs progress to lead development stages, further potency increases are carefully coordinated with appropriate optimization of clearance.

This strategy does not advocate pursuing compounds with the goal of lower fup/fumic ratios or increasing fumic as protein binding affects multiple ADME parameters,

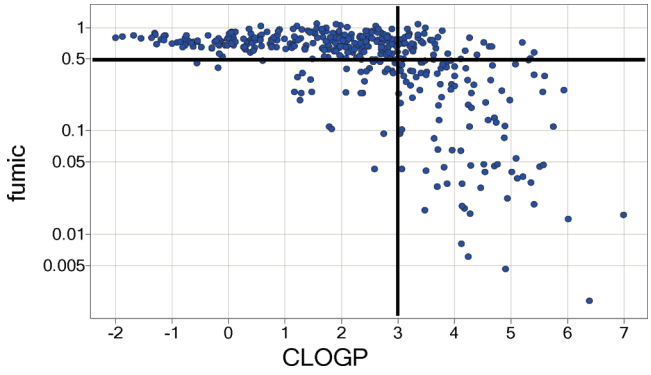

Figure 3. Plot of fumic vs cLogP. Most compounds with fumic ≥ 0.5 have cLogP ≤ 3.

making it an inappropriate end point to optimize. However, this strategy does benefit from compounds with higher fumic. A plot of fumic for these 416 compounds against cLogP (Figure 3) shows that the majority of compounds with fumic ≥0.5 have cLogP ≤ 3, which is in line with previous observation.[9] There is currently a drive to focus on compounds with cLogP ≤ 3 as these compounds show reduced propensity for in vivo toxicity.[10] A focus on chemical space with lower lipophilicity complements well with the current proposed strategy of considering compounds with higher CLia.

In summary, this analysis of 416 disparate compounds with experimentally determined plasma and microsome protein binding reveals dissimilar fup and fumic values, resulting in a significant number of compounds with a fup/fumic ratio ≤0.4. This subunity ratio provides an opportunity to consider compounds with higher CLia without increasing the calculated hepatic extraction (Er). However, without an offset increase in apparent dose, consideration of higher CLia compounds is balanced by a requisite increase in potency, which is proportional for compounds with high fumic. Hence, in early discovery, the benefit of considering higher CLia compounds with appropriate integration of fup/fumic ratios and values is a potential increase in chemistry space for evaluation. While the effect of higher clearance on dose may initially be addressed by monoparametric improvement in potency, a necessary optimization during the course of a program, ultimate success is dependent on coordinated multiparametric progression of both potency and clearance.

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DOI: 10.1021/ml900012h | *ACS Med. Chem. Lett.* **2010**, *1*, 50–53

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ACKNOWLEDGMENT We thank Theodore Liston for his critical and insightful comments on the manuscript.

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DOI: 10.1021/ml900012h | *ACS Med. Chem. Lett.* **2010,** *1*, 50–53