Discrepancy between *in vitro* potency and *in vivo* efficacy in human - Implications in PK-PD modeling

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BACKGROUND

- The free drug hypothesis that only the unbound (free) drug molecules exert effects by binding to targets has been one of dogmas of pharmacology.
- Quantitative prediction of efficacious exposures (AUC, Cmin *etc*) using *in vitro* potency (i.e., IC_{50}) and unbound fraction has been practiced widely by modelers or other researchers at early discovery or preclinical drug development.
- However, its fundamental assumption that the *in vitro* potency is well correlated with *in vivo* efficacy has never been verified extensively. Thus, we tried to look into this assumption by searching a wide range of published data.
- If the free drug hypothesis is valid and *in vitro* potency measurements are well correlated with *in vivo* effects, patients' exposure to unbound drug (e.g., steady state unbound average concentrations, $C_{u,ss,avg}$) accomplished by the approved dosage regimens should be higher than or, at least, comparable to the *in vitro* potency parameters such as IC_{50}, EC_{50}, K_i *etc*.

METHOD

- As this relationship has never been examined widely for currently-used drugs, we reviewed the ratios of $C_{u,ss,avg}$ / potency for drugs of major therapeutic categories using the PK and *in vitro* potency information published to journals. (Ideally, the ratios of all drugs should be >> 1.)
  - ❖ PK parameters (F, CL, Vd) of each drug and its typical approved dosage regimen were used to calculate the $C_{u,ss,avg}(= f_u \cdot F \cdot Dose/(CL \cdot \tau) = f_u \cdot AUCss/\tau)$.
  - ❖ When therapeutic dose AUCs reported in patients were available, they were chosen over the calculated $C_{u,ss,avg}$.
  - ❖ As for the potency data, those for at least two moieties in the same class obtained by a single research team and reported in a single original research article were used so that inter-laboratory or inter-method variation may be avoided.
- The 49 drug moieties' (13 categories) potency data collected by the above criteria are listed in the **Table 1.** Antibiotics, diuretics, NSAIDs and COX_2 inhibitors were not included because they were not appropriate to apply the ratio calculation method used herein.

RESULTS

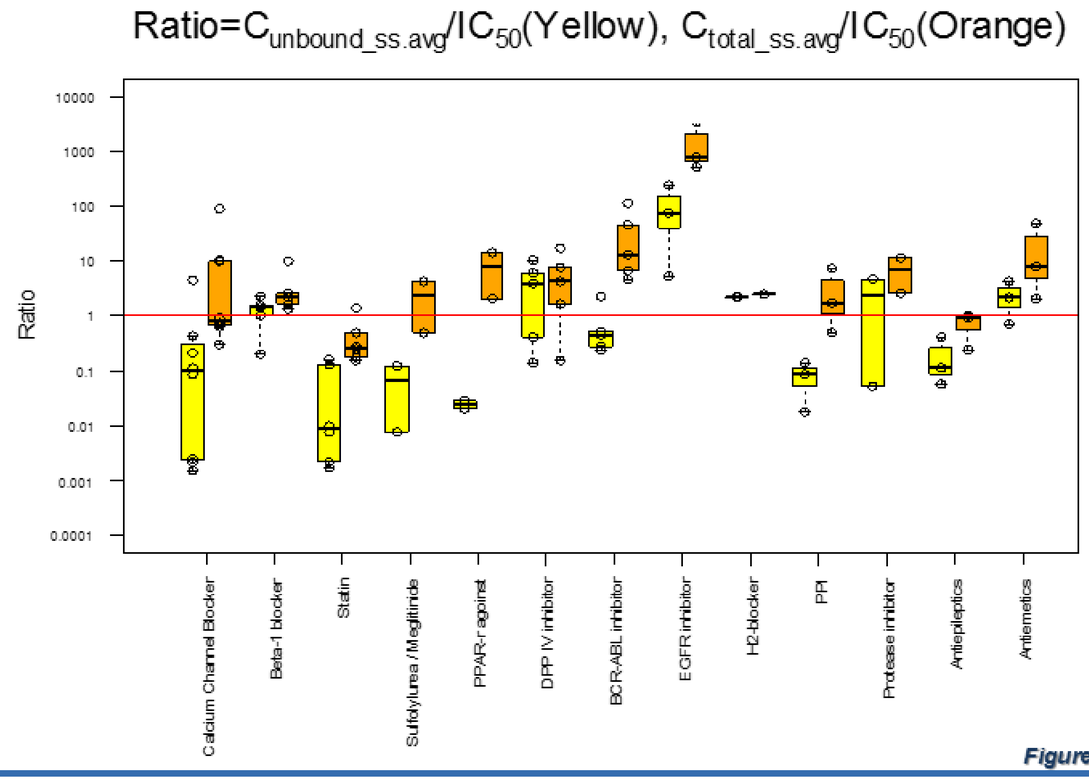

Figure 1. Distribution of the $C_{u,ss,avg}$ / potency and $C_{tot,ss,avg}$ / potency ratios calculated using approved dosage regimens and their potency data (Table 1)

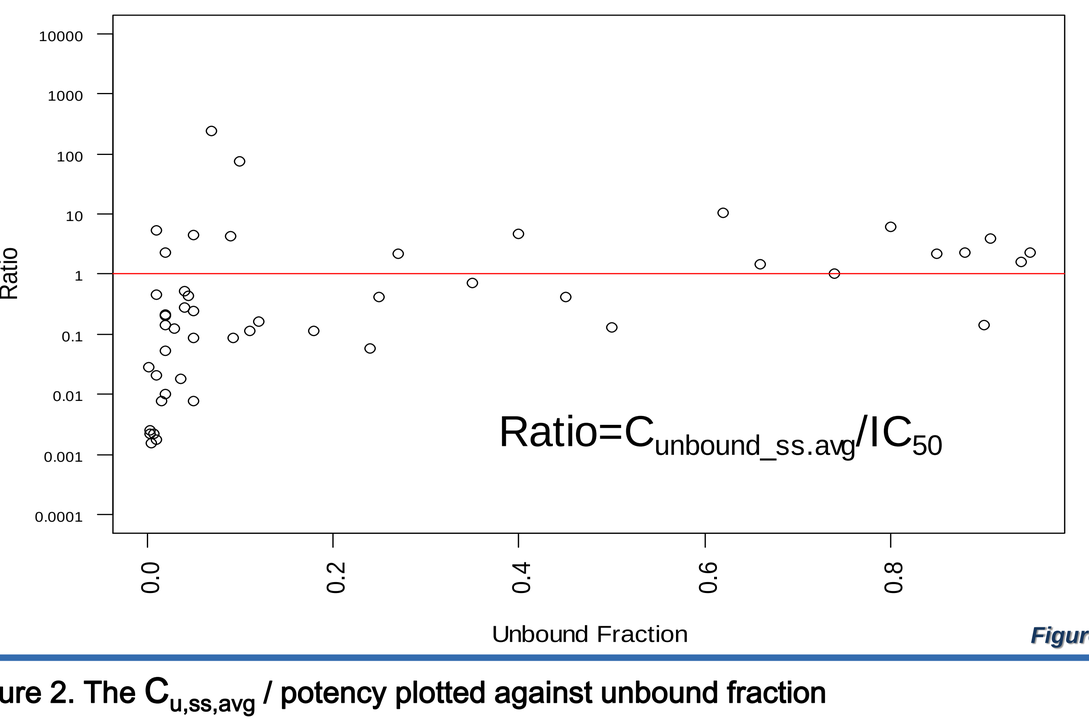

Figure 2. The $C_{u,ss,avg}$ / potency plotted against unbound fraction

Table 1. Drug classes used to calculate the $C_{u,ss,avg}$ / potency ratios.

Class	Name	Ref.	Class	Name	Ref.
CCB	Nitrendipine	JPET 1995;274:419-426 (rat tail artery contraction)	DPP IV inhibitor	Alogliptin	(DPP-4 extracted from Caco-2)
	Felodipine			Saxagliptin	
	Nimodipine			Vildagliptin	
	Nisoldipine		BCR-ABL inhibitor	Dasatinib	Am J Hematol 2012;87(11): E125-E128 (wild type BCR-ABL)
	Nifedipine			Ponatinib	
	Amlodipine			Nilotinib	
	Verapamil			Bosutinib	
	Diltiazem			Imatinib	
Beta blocker	Carbedilol	Br J Pharmacol 2005;144: 317–322 (CHO-K1 cell, Kd value)	EGFR inhibitor	Gefitinib	Genes to Cells 2013;18:110–122 (kinase assay)
	Bisoprolol			Erlotinib	
	Metoprolol			Lapatinib	
	Atenolol		H2-blocker	Ranitidine	Scand J Gastroenterol 1985;20(8):917-921
	Acebutolol			Cimetidine	
Statin	Rosuvastatin	Am J Cardiol 2001;87:5A: 28B-32B (human liver microsome)	PPI	Omeprazole	Physiol Paris 2000;94(1):19-23
	Atorvastatin			Pantoprazole	
	Cerivastatin			Rabeprazole	
	Simvastatin		Protease inhibitor	Indinavir	*
	Fluvastatin			Saquinavir	
	Pravastatin		Antiepileptics	Phenytoin	JPET 1993;266:829-835 (mice neuroblastoma cell, current)
Sulfonyurea	Repaglinide	Diabetes 2002;51:2789–2795 (current inhibition)		Lamotrigine	
	Nateglinide			Carbamazepine	
PPAR-r agonist	Rosiglitazone	PNAS 2001;98(24): 13919–13924 (EC50)	Antiemetics	Ramosetron	JPET 1992;263(3):1127-1132 (neuroblastoma cell, Ki)
	Pioglitazone			Granisetron	
DPP IV inhibitor	Linagliptin	JPET 2008;325:175–182		Ondansetron	
	Sitagliptin			-	

JPET: J Pharmacol Exp Ther
PNAS: Proc Nat Acad Sci
*4th International Workshop on Clinical Pharmacology of HIV Therapy, 27–29 March 2003

- In the 49 moieties, the $C_{u,ss.avg}$ / potency ratios were <1 in 32 (65%) and <0.1 in 15 (31%) moieties. Even in the case of $C_{tot,ss.avg}$ (total concentration), the ratios of 15 (31%) moieties were <1. Average ratios (unbound) of statins and CCBs were lower than 0.1.
- If the free drug hypothesis is valid, discrepancy between the *in vitro* potency and *in vivo* effect is the only suspect of the "ratio<1" phenomena. If such discrepancies are so widespread, we had better be conservative in interpreting the *in vitro* data.
  - ❖ All of the currently-used methods assaying the *in vitro* potency should be collectively regarded as mere ancillary, screening tools that cannot be used to infer the *in vivo* efficacy.
- The finding that the ratio<1 phenomena are most common at drugs with f_u < 0.05 (Figure 2) implies that there still remains room for improvement in protein binding assays, especially at the higher extreme.

CONCLUSION

- Because the *in vitro* potency varies according to assay methods and laboratories, the ratio of each drug herein may not be a solid one. However, the trend observed across the 13 classes suggests us that the traditional, free drug hypothesis-based approaches are not desirable when we have *in vitro* data only.
- Predicting human efficacious concentrations with the protein binding and *in vitro* potency data should not be a standard approach any more (especially for molecules with high protein binding: f_u < 0.05), despite old respects on the free drug hypothesis.

- **Before comparing the *in vivo* effects in animals, the fate of candidates should not be determined by the f_u and *in vitro* potency data only.**