Plasma Protein Binding: Friend or Foe in Drug Discovery?

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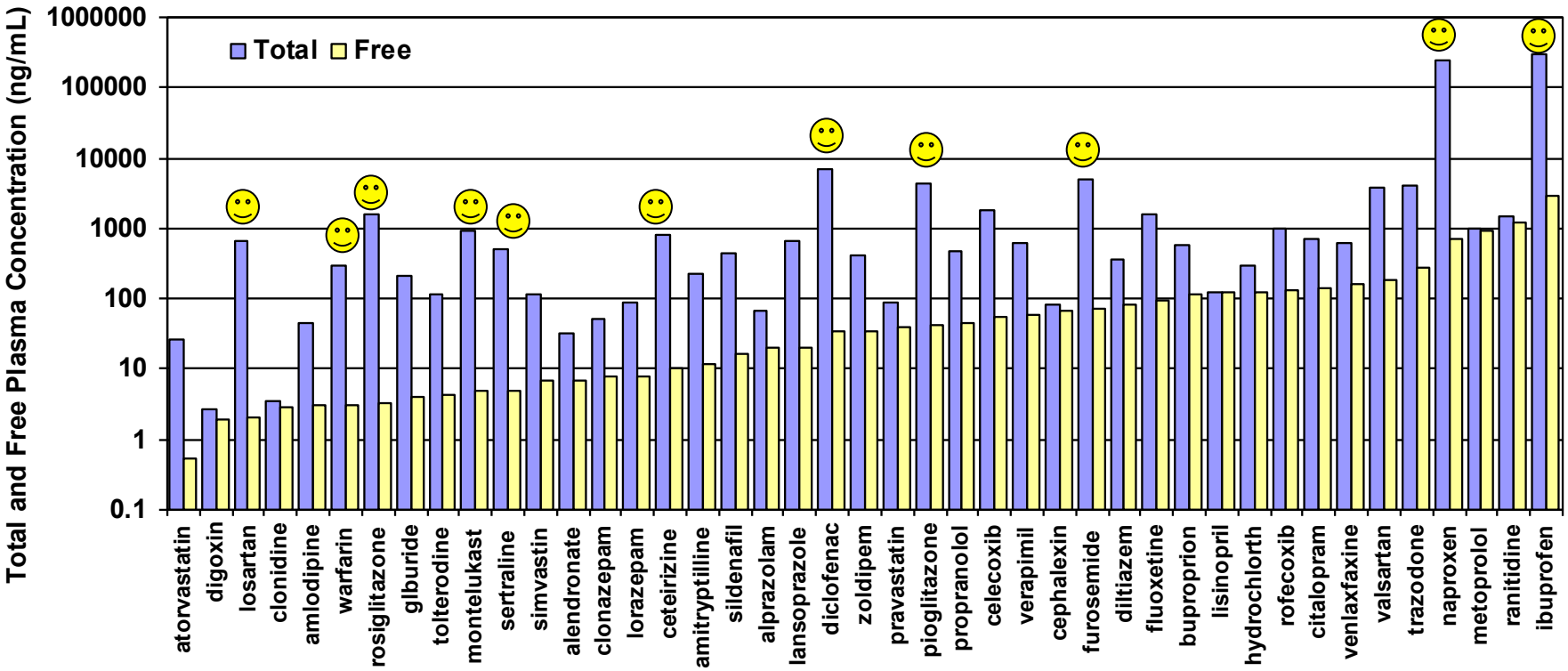
Plasma Protein Binding of Some Top 100 Most Prescribed Drugs



☺ Drugs with 2 log units between total and free plasma concentration, percent bound greater than 98-99.9 %.

If ppb drives dose, why are these compounds successful drugs?

Smith, D.A. et. Al. *Nat. Rev. Drug Disc.* 2010, *9*, 929-939.

Individual Drugs

- Fluconazole
- f_u = 0.88
- Identical free drug concentration (SD or MD)
  - Plasma
  - CSF, Saliva
  - Vaginal secretions
  - Breast milk, sputum
  - Prostatic seminal vesicle fluid

- Naproxen
- f_u = 0.003
- Identical free drug concentration (SD or MD)
  - Plasma
  - Synovial fluid (deep tissue fluid)

Suggests high plasma protein binding is not a hindrance to distribution or success

Importance of selecting your battles

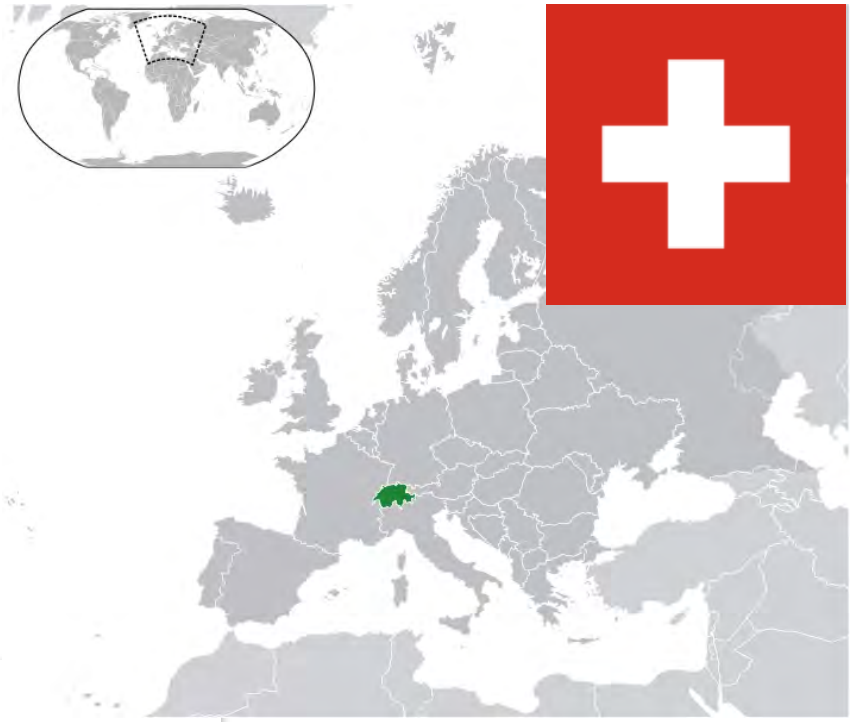
We have many foes in Drug Discovery

Toxicity
Potency
Selectivity
Intrinsic Clearance
Drug Drug Interactions
Reactive metabolites
Etc

Why pick a fight with a neutral agent?

Attacking ppb is like invading Switzerland!

No benefit and a whole heap of trouble!



Plasma Protein Binding is a Neutral Parameter

Plasma protein binding is a key parameter to measure

- Important to unmask free concentration
- Important in PK and dose prediction

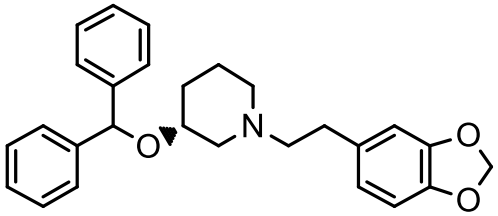
Plasma protein binding is **not** a key parameter to modulate

- Neutral on free concentration
- Neutral on half-life*
- Neutral on dose

Exception is when Vd is fixed to blood volume*

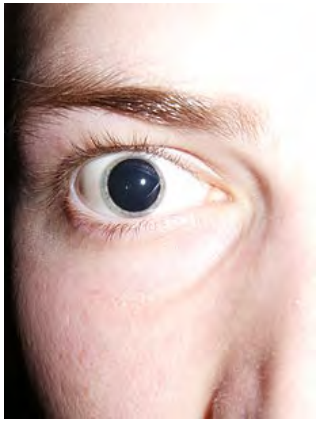
Free drug concentrations and antimuscarinic activity of zamifenacin

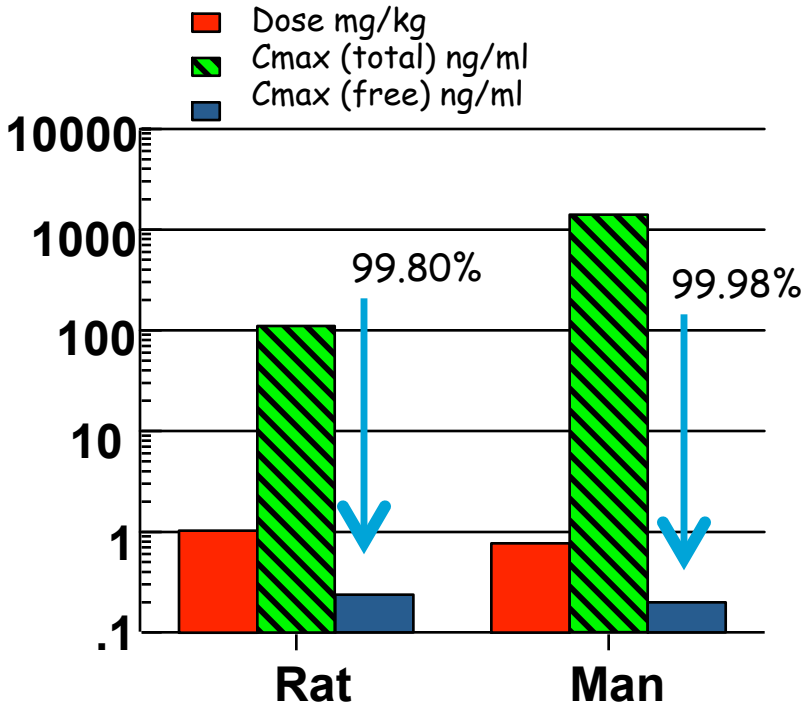
Zamifenacin, potent and selective M3 muscarinic antagonist.



Translatable Biomarker

Inhibition of pupillary response to light (rat) or transient blurred vision (man)





10-fold difference in free fraction,
0.002 and 0.0002 respectively
Highly bound in both species
Free concentration driving effect is identical

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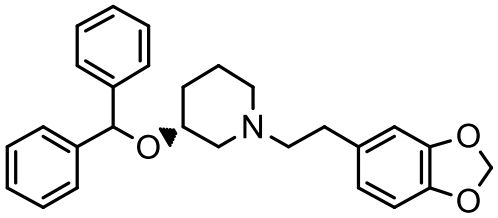
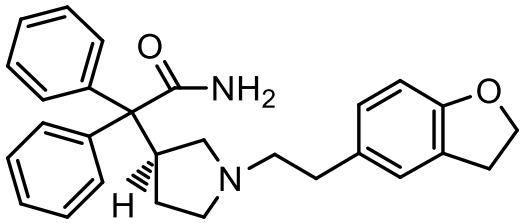
Beaumont et al, 1994, Brit J. Clin. Pharm. 38, 179P

Comparison of M3 Muscarinic Receptor Antagonists



Zamifenacin



Darifenacin

Compounds	Zamifenacin / Darifenacin
In Vitro K_b (nM)	2 fold
Intrinsic Clearance	Similar
F_u	300 fold (0.06 vs. 0.0002)
Daily Clinical Dose	2 fold

Dose depends on potency and intrinsic clearance, but not PPB

Dose - Steady State Equation

$$\frac{\overbrace{\text{Dose}}^{\text{Objective}}}{\tau} = \frac{\overbrace{C_{ss,\,avg}}^{\text{Potency}} \bullet \overbrace{CL}^{\text{Clearance}}}{\underbrace{F}_{\text{Absorption + CL}}}$$

Wagner, *Nature* (1965)

Determinants of Dose

$$\underbrace{\frac{\text{Dose}}{\tau}}_{\text{Objective}} = \underbrace{\frac{C_{ss,\,avg} \bullet CL}{F}}_{\substack{\text{Potency} \\ \text{Clearance}}} \qquad \underbrace{}_{\text{Absorption + CL}}$$

Wagner, *Nature* (1965)

Determinants of Dose – Oral drugs, hepatically cleared

$$\underbrace{\frac{\text{Dose}}{\tau}}_{\text{Objective}} = \frac{\overbrace{C_{ss,\,avg}}^{\text{Potency}} \bullet \overbrace{CL}^{\text{Clearance}}}{\underbrace{F}_{\text{Absorption + CL}}}$$

Wagner, *Nature* (1965)

For hepatically cleared drugs dosed orally

$$CL = \frac{Qh \bullet (fu \bullet CLint)}{Qh + (fu \bullet CLint)}$$

Pang, *J Pharmacokin Biopharm* (1973)

$$F = fa \bullet \left(1 - \frac{CL}{Qh}\right)$$

Determinants of Dose – Oral drugs, hepatically cleared

Objective: $\frac{Dose}{\tau}$ = Potency: $C_{ss,avg}$ • Clearance: $\frac{CL}{F}$ (F = Absorption + CL)

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Substitute terms to reveal protein binding influence

$$Dose = \frac{\left(\frac{Css,u}{fu}\right) \bullet \left(\frac{Qh \bullet fu \bullet CLint}{Qh + (fu \bullet CLint)}\right) \bullet \tau}{fa \bullet \left(1 - \frac{\left[\frac{Qh \bullet fu \bullet CLint}{Qh + (fu \bullet CLint)}\right]}{Qh}\right)}$$

Substitute and simplify

Cancel fu and Qh terms

$$Dose = \frac{(Css,u) \bullet \left(\frac{Qh \bullet CLint}{Qh + (fu \bullet CLint)}\right) \bullet \tau}{fa \bullet \left(1 - \left[\frac{fu \bullet CLint}{Qh + (fu \bullet CLint)}\right]\right)}$$

Simplify denominator

$$Dose = \frac{(Css,u) \bullet \left(\frac{Qh \bullet CLint}{Qh + (fu \bullet CLint)}\right) \bullet \tau}{fa \bullet \frac{Qh + (fu \bullet CLint) - fu \bullet CLint}{Qh + (fu \bullet CLint)}}$$

Consolidate numerator and denominator

$$Dose = (Css,u) \bullet \left(\frac{Qh \bullet CLint}{Qh + (fu \bullet CLint)}\right) \bullet \tau \bullet \frac{Qh + (fu \bullet CLint)}{fa \bullet Qh + (fu \bullet CLint) - fu \bullet CLint}$$

simplify

$$Dose = \left(\frac{Css,u \bullet CLint \bullet \tau}{fa}\right)$$

"the chemists most important pharmacokinetic equation"

No requirement for f_u

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Consolidate numerator and denominator

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simplify

$$Dose = \left(\frac{Css,u \bullet CLint \bullet \tau}{fa}\right)$$

"the chemists most important pharmacokinetic equation"

No requirement for f_u

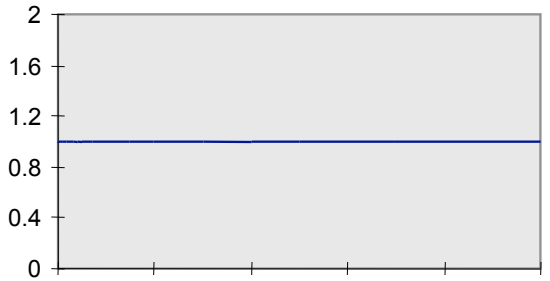
"The only determinants of unbound steady state exposure are intrinsic clearance and dose"
- Harold Boxenbaum

"Plasma protein binding alone cannot be regarded as either a positive or negative aspect of a compound" - van de Waterbeemd, *J Med Chem* (2001)

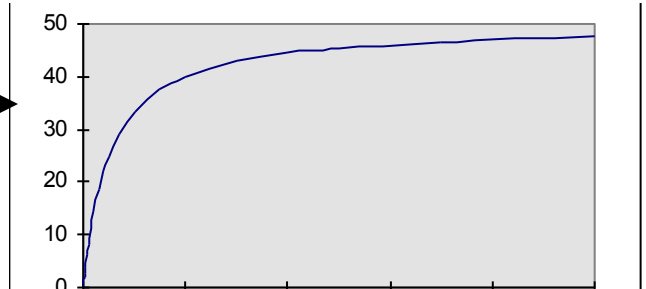
Thought Experiment 1 –Modulate Fraction Unbound, Maintain Dose and Intrinsic Clearance (unbound)

Cl_i^u = 1000 mL/min/kg, Q = 50 mL/min/kg, Complete absorption, Fixed dose

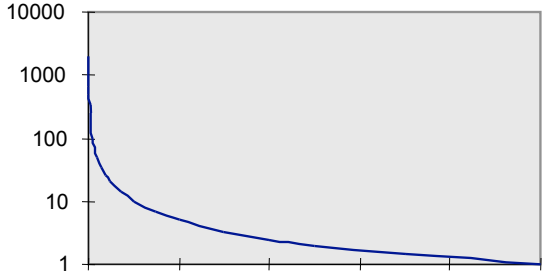
$AUC_u = dose / Cl_u$



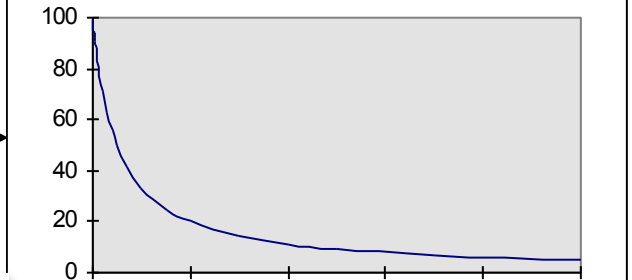
$Cl_H = Q(f_u.Cl_i^u)/(Q+f_u.Cl_i^u)$



$AUC_{total} = dose/Cl$



$F = 1 - (Cl_H/Q)$

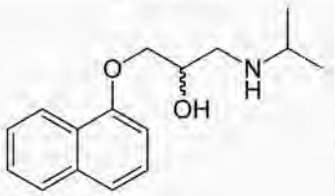


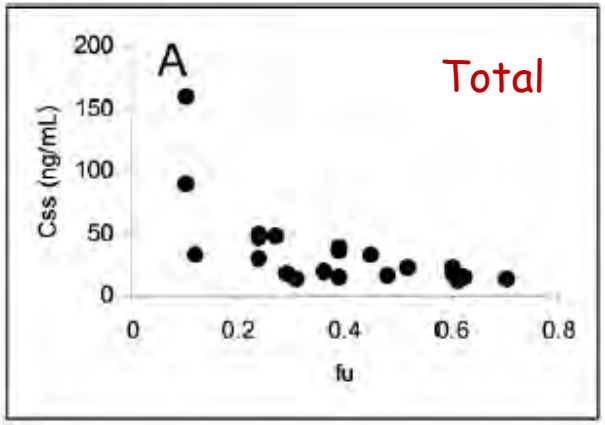
Math courtesy of Don Walker

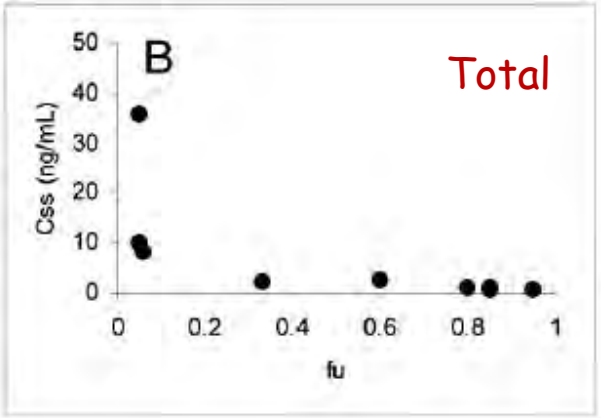
It is tough to modulate fraction unbound - IPRL

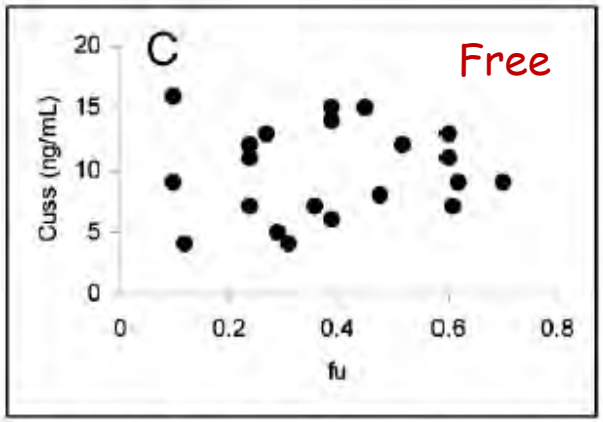
Isolated perfused rat liver model: modulate ppb with amount of BSA in perfusate

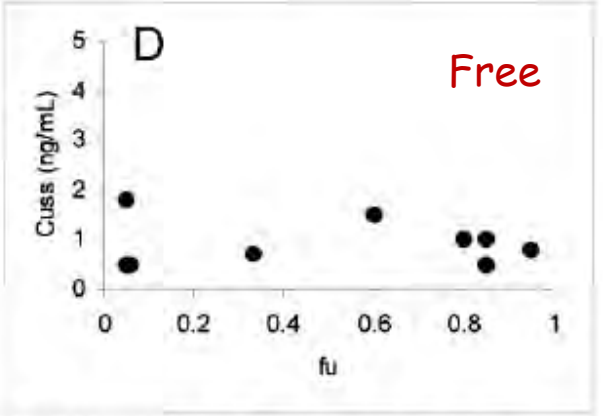
Propranolol











Taurocholic acid

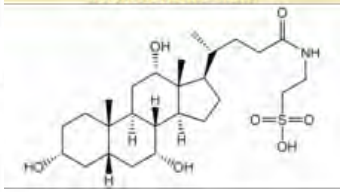


Fig. (3). Effect of protein binding on the steady state total (**A** and **B**) and unbound (**C** and **D**) concentration of propranolol (**A** and **C**) and taurocholate (**B** and **D**) in isolated perfused rat liver model. An increase of f_u leads to a reduction of total concentration but no change for the unbound concentration. Data for propranolol and taurocholate were from Jones *et al.* and Smallwood *et al.*, respectively [30, 31].

In vivo: C_{tot} decreases with increase in f_u but C_{free} does not change

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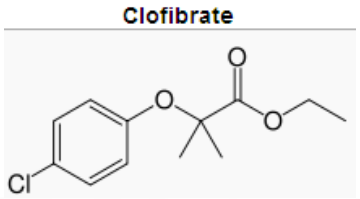
Liu, X. et. Al. *Curr. Top. Med Chem.* 2011, *11*, 450-466.

It is tough to modulate fraction unbound – NAR Rats

Nagase analbuminemia rats (NAR): mutant strain from wild type Spraque-Dawley rats (less albumin)



Total

Free

3X

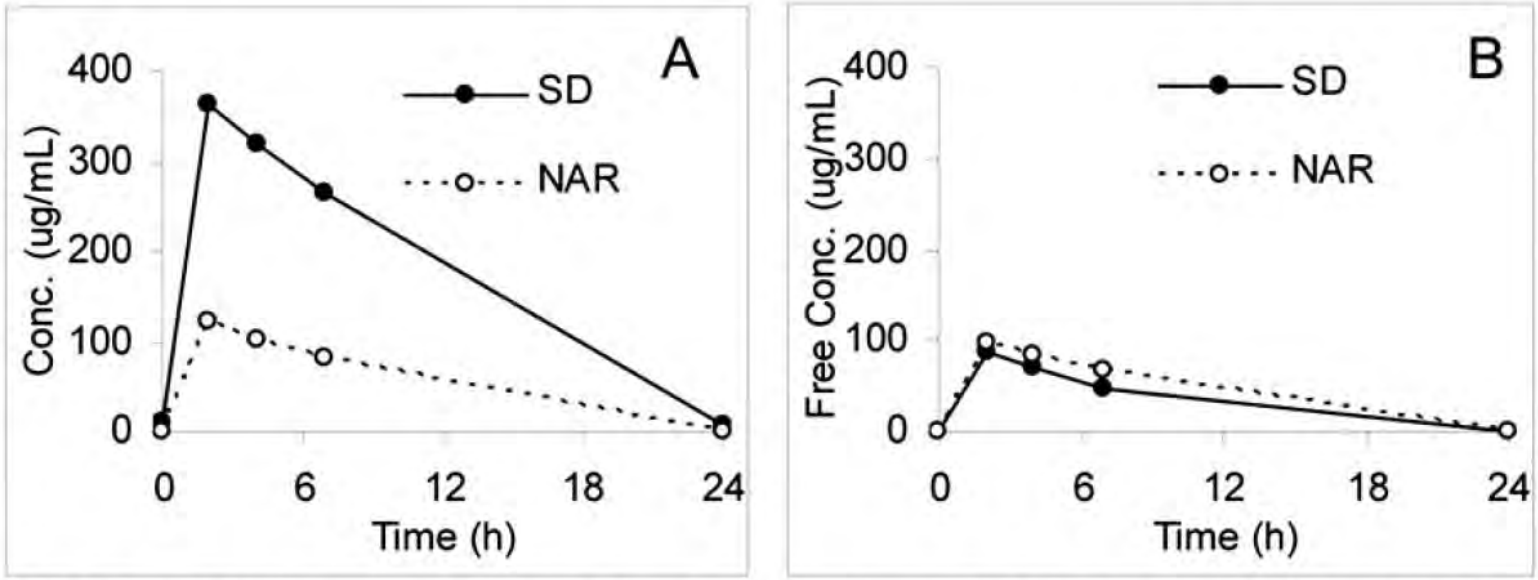


Fig. (4). Effect of protein binding on the total (**A**) and unbound (B) plasma concentration of clofibrate in Sprague-Dawley (SD, solid lines) and Nagase analbuminemia (NAR, dashed lines) rats after 4-day repeated daily oral dose of clofibrate 200 mg/kg. Total concentrations in analbuminemia rats (low plasma protein binding or high unbound fraction) were approximately 3-fold of the normal rats (high protein binding or low unbound fraction) but the unbound concentrations were similar between the two strains of rats. Data are from Miida *et al.* [34].

Decrease in f_u does not change the $Conc_u$ ONLY the $Conc_{tot}$

Liu, X. et. Al. *Curr. Top. Med Chem.* 2011, *11*, 450-466.

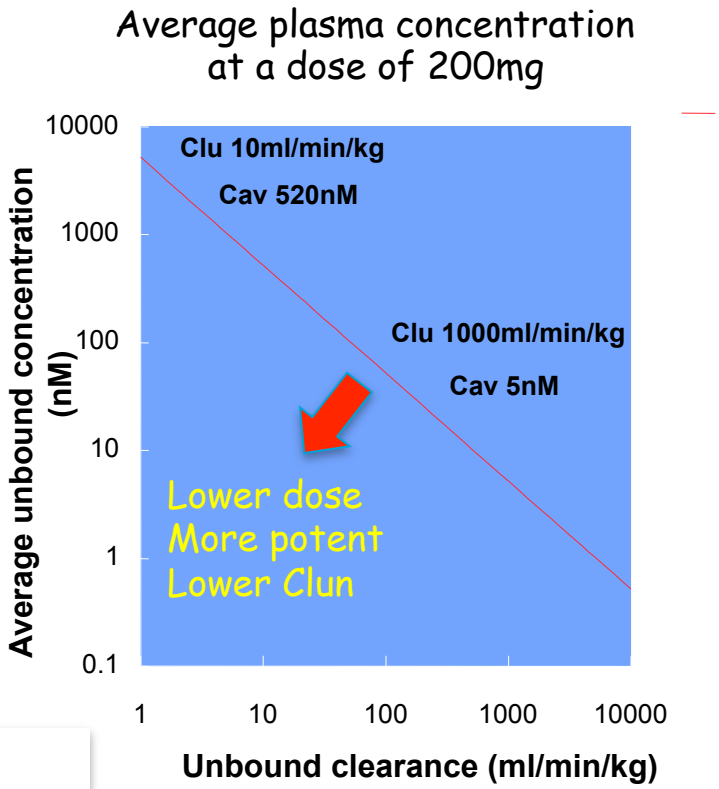
Modulating Dose
- Unbound Clearance and Potency (unbound concentration)

$$\text{Dose} = \frac{C_{ss,avg} \bullet CL \bullet \tau}{F}$$

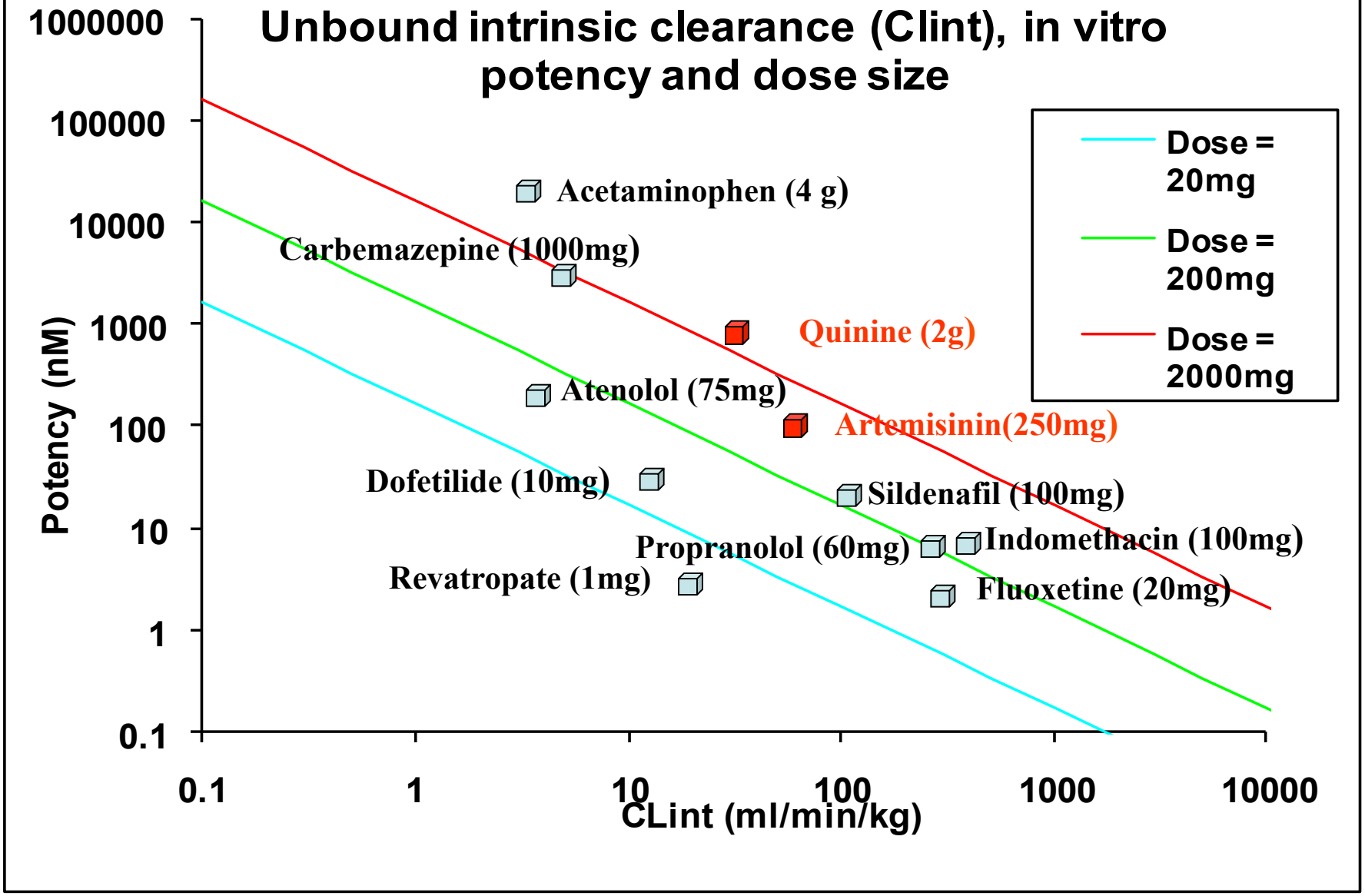
τ is dose interval

Css is steady state Cav (unbound)
Related to potency

Cl is the clearance of the compound (unbound)
Related to metabolism/excretion rate



Modulating Dose
- Dose calculation for marketed drugs

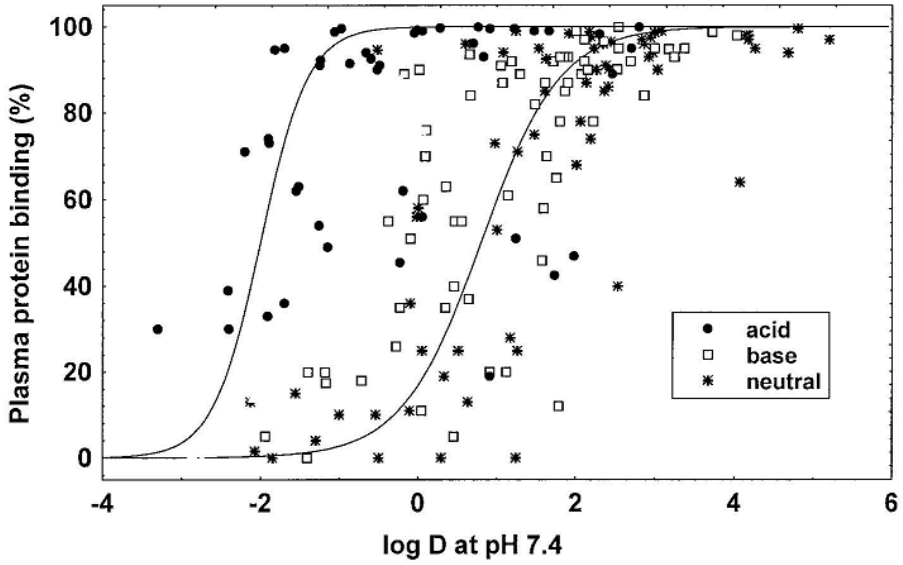


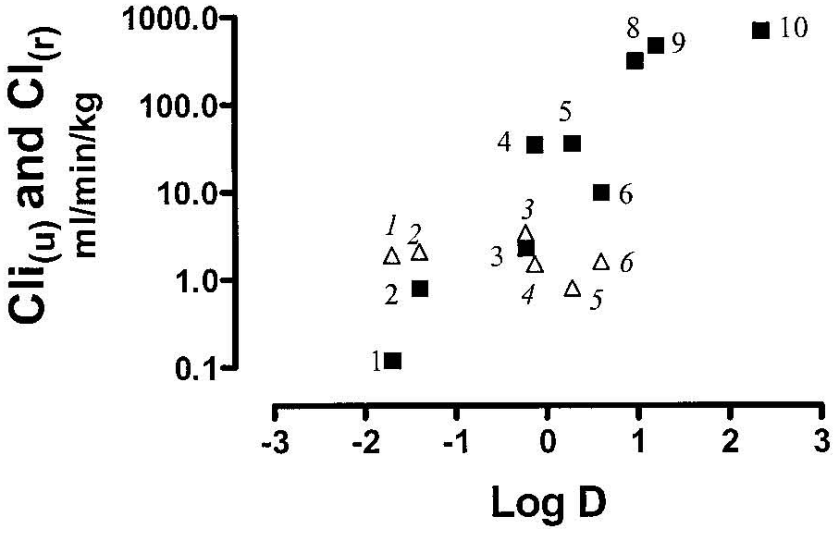
A Conundrum Wrapped up in a Riddle

Why is the literature replete with approaches that target lower plasma protein binding?

The Paradox:

The tactics to modulate fraction unbound are the same as those to modulate unbound intrinsic clearance





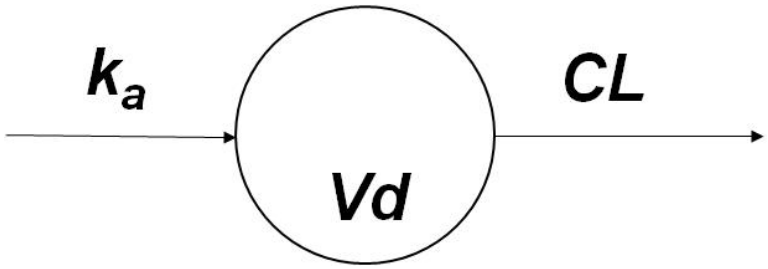
A strategy to lower plasma protein binding by lowering log $D_{(7.4)}$ may appear to improve dose……

…… **but only by lowering unbound intrinsic clearance!!**

van de Waterbeemd, *J Comp Aid Mol Des* (2001)

Unless we want to make life complicated!

Compartmental Model:



Physiological Parameter Models:

$$CL = \frac{Qh \times CLint \times fu_{plasma}}{Qh + CLint \times fu_{plasma}} \quad \text{or} \quad GFR \times fu_{plasma}$$

$$Vd = Vc + Vt\left(\frac{fu_{plasma}}{fu_{tissue}}\right)$$

$$F = fa \times \left(1 - {}^{CLh}/_{Qh}\right)$$

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Target Exposure:
5nM as $C_{u,ss,max}$, $C_{u,ss,min}$ or $C_{u,ss,avg}$

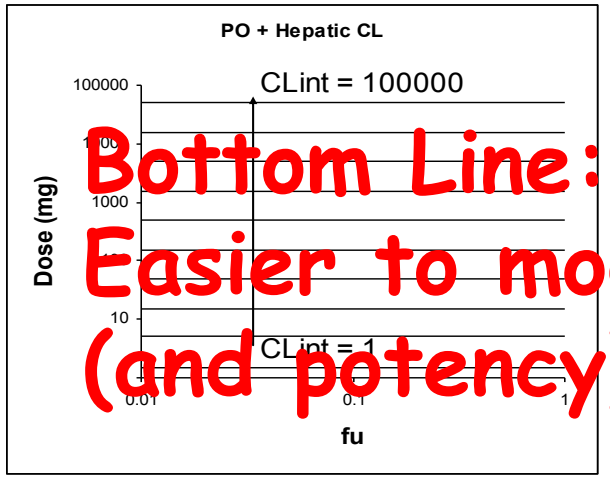
Primary parameters:
fu: 0.01 – 1
CLint: 1 – 100,000 ml/min/kg
fa=1
Tau=24 hrs
ka=1.8 hr^{-1}
fu_{tissue}=0.01
Vc = 0.2 L/kg (extracellular fluid)
Vt = 0.4 L/kg (intracellular fluid)

Secondary parameters:
Vd = 0.6 – 40 L/kg
CL:0.01 – 19.996 ml/min/kg
$t_{1/2}$: 1 – 800 hr

Dose to Achieve Target Cav

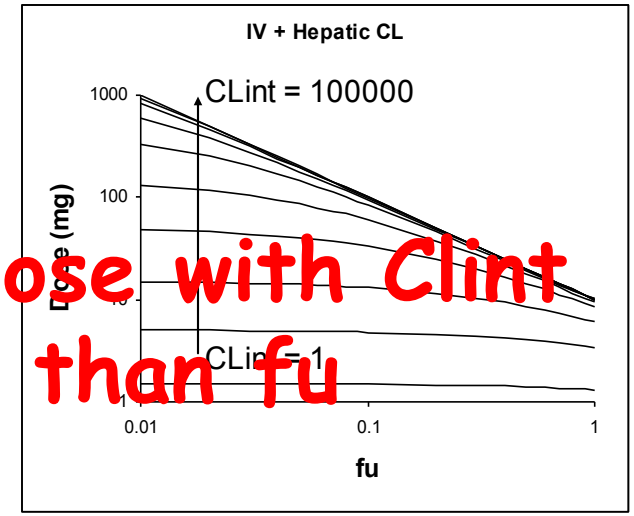
$$\text{Dose} = \frac{\text{Css,avg} \bullet \text{CL} \bullet \tau}{\text{F}}$$

Hepatic / PO



$$\text{Dose} = \frac{\text{Css,avg} \bullet \text{CL} \bullet \tau}{1}$$

Hepatic / IV



Bottom Line: Easier to modulate dose with Clint (and potency) rather than fu

As fu ↑ Css,av ↓ but is offset by Cl ↑ and F ↓

Overall, dose is independent of fu

As fu ↑ Css,av ↓ but is only partially offset by Cl ↑

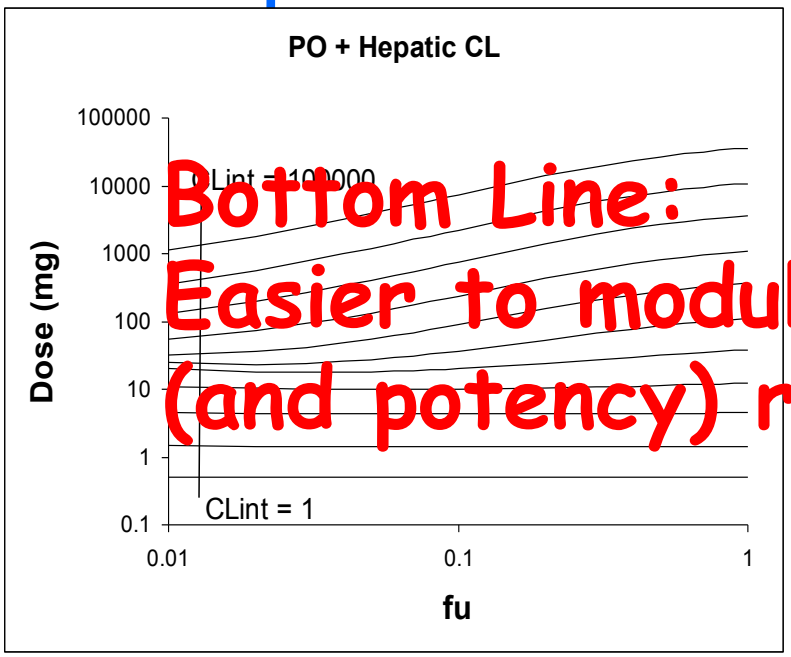
Effect is only seen at high values of intrinsic clearance

Math courtesy of Tristan Maurer

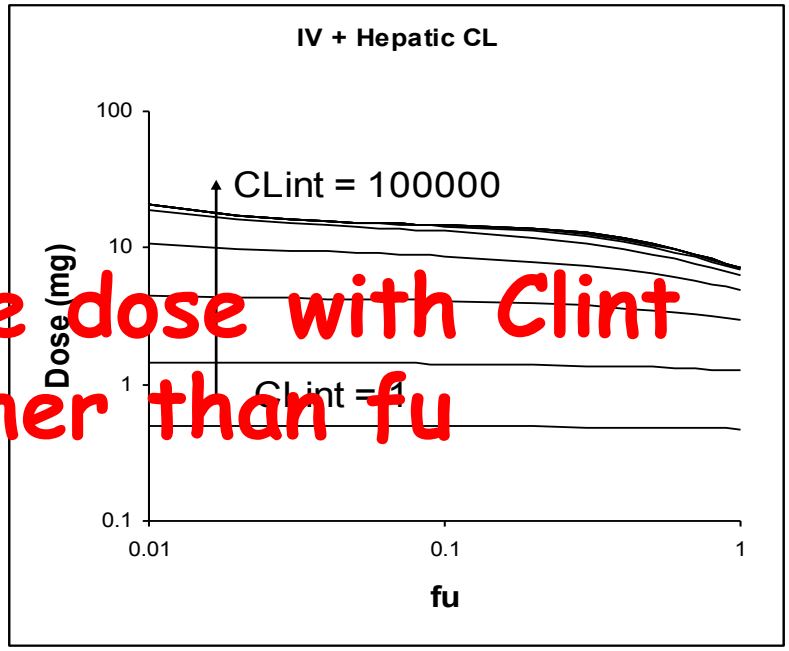
Dose to Achieve Target Cmax

$$\text{Dose} = \frac{\text{Cmax}}{F} \times Vd \times (1 - e^{-k_{el} \times \tau}) \times e^{k_{el} \times t_{max}}$$

Hepatic / PO



Hepatic / IV



Bottom Line:
Easier to modulate dose with Clint (and potency) rather than fu

As fu ↑ Vd and Cl ↑ but Cmax and F ↓
Increased dose requirements only observed at high Clint due to nonlinear effects on Cl and F

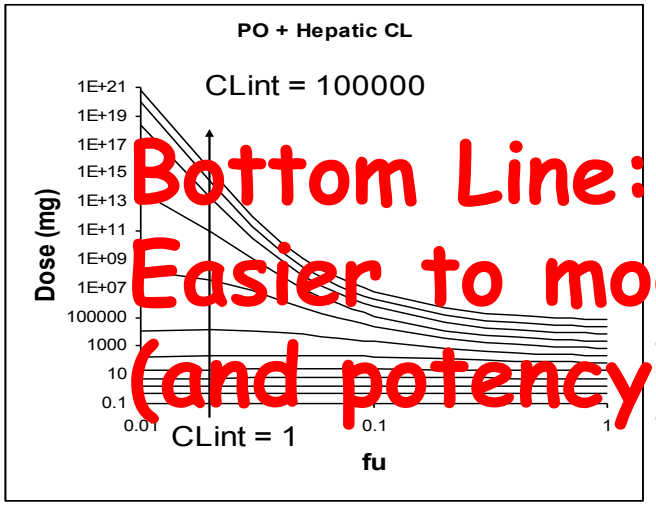
As fu ↑ Vd and Cl ↑ but Cmax ↓
Decreased dose requirements with fu only observed at high Clint

Math courtesy of Tristan Maurer

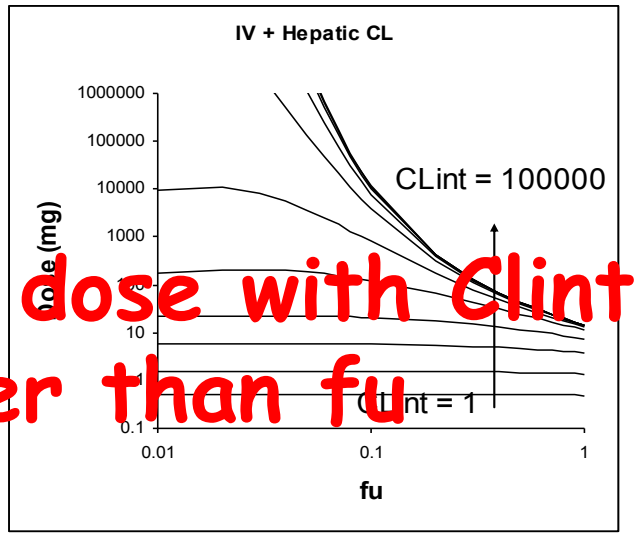
Dose to Achieve Target Cmin

$$\text{Dose} = \frac{\text{Cmin} \bullet \text{Vd} \bullet \left(k_{el} - k_a\right) \bullet \left(1 - e^{-k_{el} \times \tau}\right)}{F \times k_a \times \left(e^{-ka \times \tau} - e^{-kel \times \tau}\right)}$$

Hepatic / PO



Hepatic / IV



Bottom Line: Easier to modulate dose with Clint (and potency) rather than fu

Increasing fu, affects parameters the same way leading to a decrease in dose, but in a way that is nonlinearly related to CLint with a impact only at higher values of Clint and very high doses

With loss of F, the effect is less pronounced, but still evident at high Clint values

Math courtesy of Tristan Maurer

Plasma Protein Binding is a Neutral Parameter

Plasma protein binding is a key parameter to measure

- Important to unmask free concentration
- Important in PK and dose prediction

Plasma protein binding is **not** a key parameter to modulate

- Neutral on free concentration
- Neutral on half-life*
- Neutral on dose

Exception is when Vd is fixed to blood volume*

Unless you want to make it complicated!

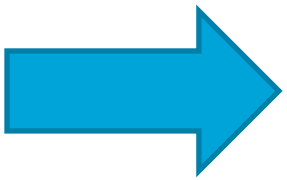
Bottom Line:
Easier to modulate dose with Clint (and potency) rather than fu

Summary and Recommendation

The next time your Discovery colleagues want to lower ppb…….

…….ask them to consider:

Dose route (iv or po)
In Vitro potency
Target (Cav, Cmax or Cmin)
Clearance route (hepatic metabolic or renal)
Clintu



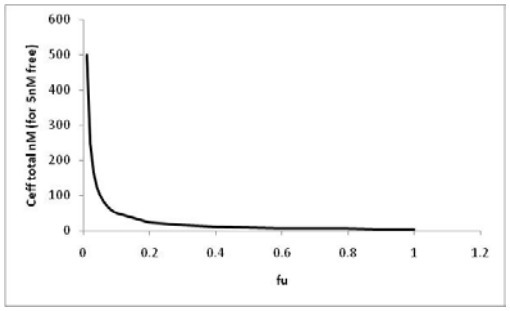
Keep calm and carry on!

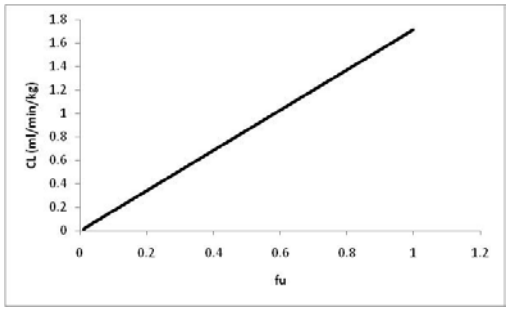
Acknowledgements

Dennis Smith
Li Di
Don Walker
Tristan Maurer

Back Ups

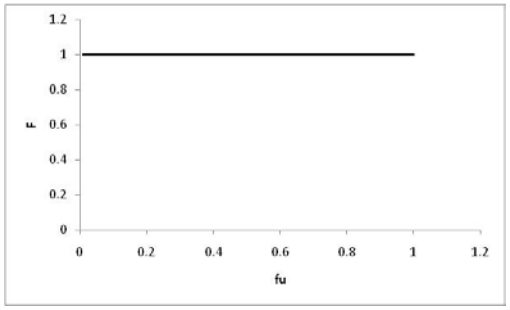
Dose to Achieve Target Cav





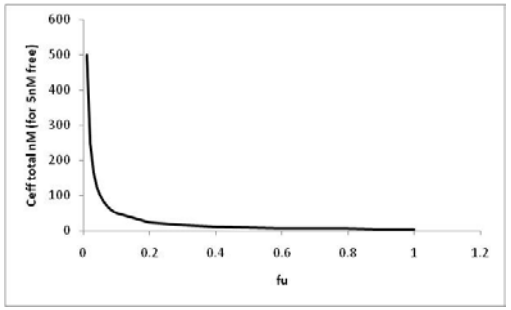
$$\frac{\text{Dose}}{\tau} = \frac{\text{Css,avg} \bullet \text{CL}}{\text{F}}$$

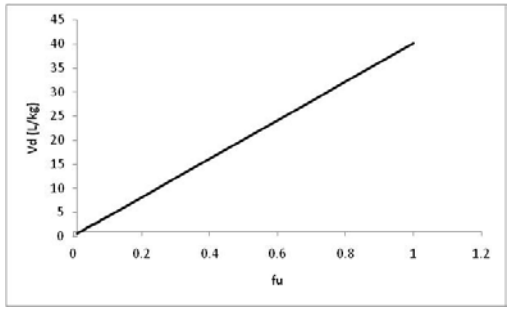
Renal PO

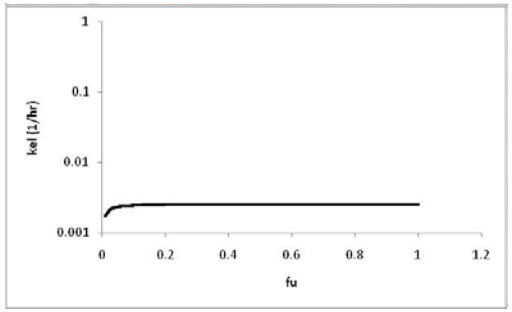


Math courtesy of Tristan Maurer

Dose to Achieve Target Cmax

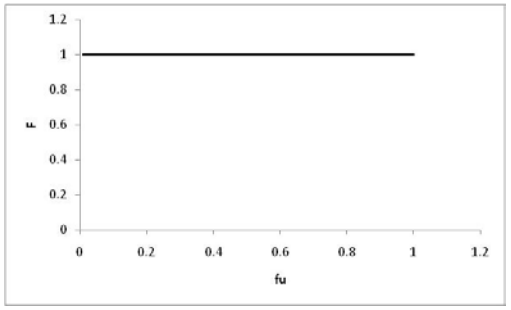






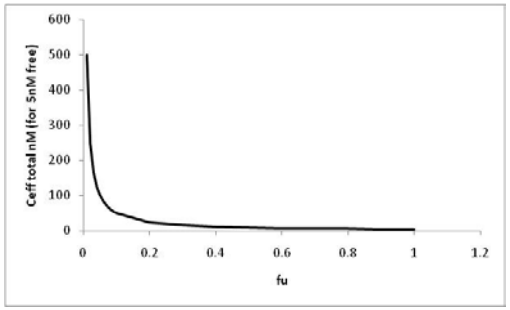
$$\text{Dose} = \frac{\text{Cmax}}{\text{F}} \times \text{Vd} \times (1 - e^{-k_{el} \times \tau}) \times e^{k_{el} \times t_{max}}$$

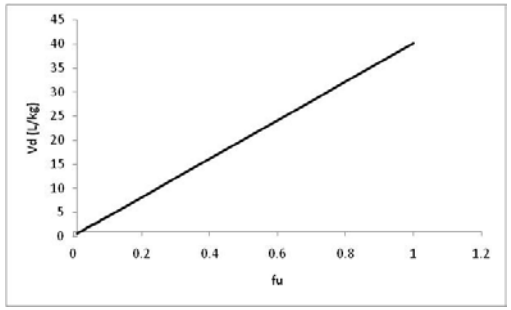
Renal PO

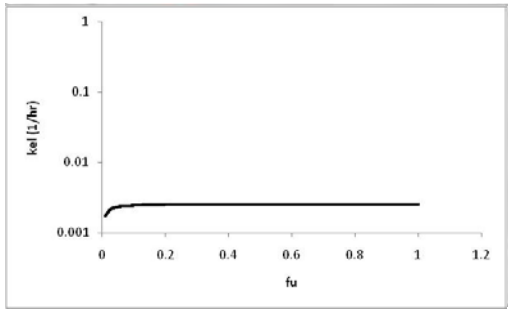


Math courtesy of Tristan Maurer

Dose to Achieve Target Cmin

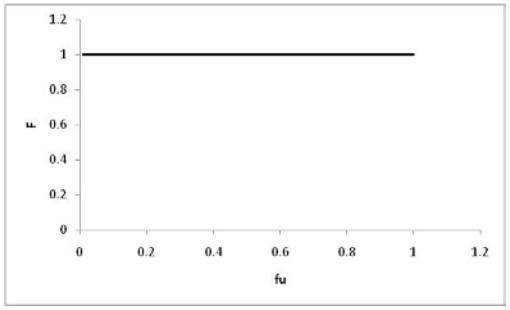






$$\text{Dose} = \frac{\text{Cmin} \bullet \text{Vd} \bullet \left(k_{el} - k_a\right) \bullet \left(1 - e^{-k_{el} \times \tau}\right)}{F \times k_a \times \left(e^{-ka \times \tau} - e^{-kel \times \tau}\right)}$$

Renal PO



Math courtesy of Tristan Maurer