Supplemental data:

Correction for non-specific binding to various components of ultrafiltration apparatus and impact on estimating *in vivo* rat clearance for a congeneric series of 5-ethyl, 5-n-alkyl barbituric acids.

Drug Metabolism and Disposition

Peter Ballard and Malcolm Rowland

Derivation of an equation to correct for non-specific binding to components of the ultrafiltration apparatus.

Consider the scheme depicting various parts of the ultracentrifuge apparatus (Supplemental Figure 1). Assume that the drug concentration is below the association constant of the protein and let $\theta = {}^{C_b}/_{Cu}$, where C_b and Cu are the bound and unbound concentrations, respectively. Now consider the events in each part of the apparatus.

1. ***Addition of protein-free drug solution to the top of the ultrafiltration reservoir, without filtration.***

Then the mass balance can be expressed as:

$$Total = V_M . C_{tot} = V_M . Cu_R + A_R \qquad \textbf{Equation A1}$$

where V_M is the volume of the added solution, C_{tot} and Cu_R are the total and unbound concentrations of compound in the reservoir, and A_R is the amount adsorbed onto the walls of the reservoir.

Let $Kp_R = {}^{A_R}/_{Cu_R}$

Then $$V_M.C_{tot} = V_M.Cu_R + Kp_R.Cu_R$$ **Equation A2**

Therefore $$fu_R = \frac{Cu_R}{C_{tot}} = \frac{V_M}{V_M + Kp_R}$$ **Equation A3**

2. ***Addition of microsomal protein solution into the top reservoir of the ultrafiltration tube without filtration.***

If non-specific binding to the reservoir is absent, then it follows that the mass balance is:

$$V_M.C_{mic} = V_M.C_{b,mic} + V_M.Cu_{mic}$$ **Equation A4**

where C_{mic}, $C_{b,mic}$ and Cu_{mic} are the total, microsomal bound and unbound drug concentrations, so that:

$$V_M.C_{mic} = V_M.\theta_{mic}.Cu_{mic} + V_M.Cu_{mic}$$ **Equation A5**

Therefore, rearranging gives: $$fu_{mic} = \frac{Cu_{mic}}{C_{mic}} = \frac{1}{1+\theta_{mic}}$$ **Equation A6**

If the drug also binds non-specifically to the reservoir then the mass balance becomes:

$$V_M.C_{mic} = V_M.C'_{b,mic} + V_M.Cu'_{mic} + A'_R$$ **Equation A7**

where the apostrophe denotes the situation where there is binding to the reservoir.

Now $\theta_{mic} = \frac{C'_{b,mic}}{Cu'_{mic}}$ and $A_R = Kp_R.Cu'_{mic}$

Therefore $$V_M.C_{mic} = \left(V_M.\theta_{mic} + V_M + Kp_R\right).Cu'_{mic}$$ **Equation A8**

Rearranging gives:

$$C_{mic} = \left[\theta_{mic} + \frac{1}{fu_R}\right].Cu'_{mic} \qquad \textbf{Equation A9}$$

So that

$$fu'_{mic} = \frac{Cu'_{mic}}{C_{mic}} = \frac{1}{\theta_{mic} + \frac{1}{fu_R}} \qquad \textbf{Equation A10}$$

However, we actually require an expression for fu_{mic}

where

$$\theta_{mic} = \frac{1}{fu'_{mic}} - \frac{1}{fu_R} \qquad \textbf{Equation A11}$$

which when substituted into Equation 10 and rearranging gives:

$$fu_{mic} = \frac{1}{1 + \left(\frac{1}{fu'_{mic}} - \frac{1}{fu_R}\right)} \qquad \textbf{Equation A12}$$

Therefore, to calculate fu_{mic}, Cu'_{mic} has to be estimated by relating it to the concentration measured after filtration (i.e. accounting for losses to membrane and collection cup).

3. ***Events after filtration.***

Consider first the loss on the membrane as unbound drug passes through it. Drug concentration in the ultrafiltrate (Cu_{UF}) is then related to Cu'_{mic} via Equation A13.

$$Cu_{UF} = Cu'_{mic}(1 - f_{mem}) \qquad \textbf{Equation A13}$$

where f_{mem} is the fraction of the filtered drug that remains on the membrane.

However, it is not possible to measure Cu_{UF} directly, but the unbound concentration in the collection cup after any binding to the cup (Cu_C) is related to Cu_{UF} via the mass balance described in Equation A14.

$$V_{UF}.Cu_{UF} = V_{UF}.Cu_C + A_C \qquad \textbf{Equation A14}$$

where V_{UF} is the volume of ultrafiltrate and A_C is the amount of drug adsorbed to the collection cup.

Rearranging Equation A14 in an analogous manner to Equation and Equation gives:

$$fu_c = \frac{Cu_C}{Cu_{UF}} = \left(\frac{V_{UF}}{V_{UF} + Kp_c} \right) \qquad \textbf{Equation A15}$$

where fu_C is the fraction of drug unbound to the collection cup and $Kp_c = {A_c}/{Cu_c}$

Therefore, substituting Equation A15 into Equation A13 gives:

$$Cu'_{mic} = \frac{Cu_C}{fu_{mem}.\, fu_C} \qquad \textbf{Equation A16}$$

where, in this case, Cu_c is the unbound fraction of drug in the collection cup after filtration. The fraction unbound to collection cup can be determined by adding protein-free media directly to the cup through Equation A17, i.e. by assuming $Cu_{UF} = C_{tot}$.

$$fu_c = \frac{Cu_c}{C_{tot}} \qquad \textbf{Equation A17}$$

Now, the fraction of drug bound to membrane can be estimated from a control filtration containing no protein in the supernatant since, under these conditions $Cu'_{mic} = Cu_R$. Therefore, substituting into Equation A16 gives:

$$fu_{mem} = \frac{Cu_c}{Cu_R.\, fu_c} \qquad \textbf{Equation A18}$$

Consequently, Cu_c can be determined in an ultrafiltrate containing protein in the sample reservoir and therefore, by assuming that the fraction of non-specific binding to sample reservoir, membrane and collection cup does not alter with barbiturate or microsomal concentration, fu_{mic} can be estimated by rearrangement of Equations A10, A12 and A16 to give:

$$fu_{mic} = \frac{1}{1 + \left[\frac{C_{mic}.\, fu_{mem}.\, fu_c}{Cu_c} - \frac{1}{fu_R} \right]} \qquad \textbf{Equation A19}$$

or $$fu_{mic} = \frac{1}{1+\left[\frac{fu_{mem}.fu_c}{fu_o} - \frac{1}{fu_R}\right]}$$ **Equation A20**

where fu_o is the observed ratio Cu_c/C_{mic}.