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COMMENTARY

Prediction of milk plasma ratio for amphoteric substances

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In 1990, Atkinson and Begg developed a set of prediction equations to estimate milk plasma ratios (*M*/*P*) of small molecules based on the physicochemical and pharmacokinetic properties of molecules such as partition coefficient ($\log_{10}P$), distribution coefficient ($\log_{10}D$), dissociation constant (pKa) and protein binding.[1] There was a good correlation between predicted and observed *M*/*P* values for selected acidic and basic drugs in the original study.[1] However, the procedure for calculating predicted *M*/*P* values for amphoteric substances was less well-defined. Because of that, a research group reported a significant underestimation of *M*/*P* values for amphoteric small-molecule drugs.[2]

As we revisited the original publication and carefully reviewed the recent report,[1,2] we learned that the poor prediction of *M*/*P* values for amphoteric drugs was due to the misemployment of pKa values. In this article, therefore, we will demonstrate the proper application of the prediction model for amphoteric small molecules.

Since the concept of the prediction model is that a lipophilic, unionized, and unbound fraction of small molecules can distribute from maternal plasma (pH 7.4) to milk (pH 7.2) (Figure 1), the set of prediction equations uses $\log_{10}P$, $\log_{10}D$, pKa, and unbound fraction (f_u). The chemical property of acids and bases is the opposite, which is that acids ionize more at a higher pH whereas bases ionize more at a lower pH. Therefore, there are two different sets of equations for acids and bases.

Step 1 is to estimate an unbound *M*/*P* concentration ratio (M_u/P_u) value using Equation 1 for bases and Equation 2 for acids.

For basic drugs[1]:

$$M_u / P_u = \frac{1 + 10^{(\mathrm{pKa}-7.2)}}{1 + 10^{(\mathrm{pKa}-7.4)}} \quad (1)$$

For acidic drugs[1]:

$$M_u / P_u = \frac{1 + 10^{(7.2-\mathrm{pKa})}}{1 + 10^{(7.4-\mathrm{pKa})}} \quad (2)$$

Step 2 is to estimate the unbound fraction in plasma ($f_{u,p}$) and in milk ($f_{u,m}$) using Equations 3 and 4. Equations 3–6 can be used for both acids and bases.[1]

$$f_{u,p} = (\text{unbound conc in plasma}) / (\text{total conc. in plasma}) \quad (3)$$

$$f_{u,m} = \frac{{f_{u,p}}^{0.45}}{\left(6.94\times10^{-4}\right)^{0.45} + {f_{u,p}}^{0.45}} \quad (4)$$

Step 3 is to estimate the partition coefficient into the lipid phase of milk (milk lipid *P*; P_{milk}) using Equation 5. Then, calculate the antilogarithm.[1]

$$\mathrm{Log}_{10}P_{milk} = 1.29\,\mathrm{Log}D_{7.2} - 0.88 \quad (5)$$

Step 4 is to estimate a constant *K* by using Equation 6 along with P_{milk} and $f_{u,m}$.[1]

$$K = \left(\frac{0.955}{f_{u,m}}\right) + \left(0.045 \times P_{milk}\right) \quad (6)$$

The last step is to calculate *M*/*P* values. Use Equation 7 for bases and Equation 8 for acids. Then, calculate the antilogarithm.

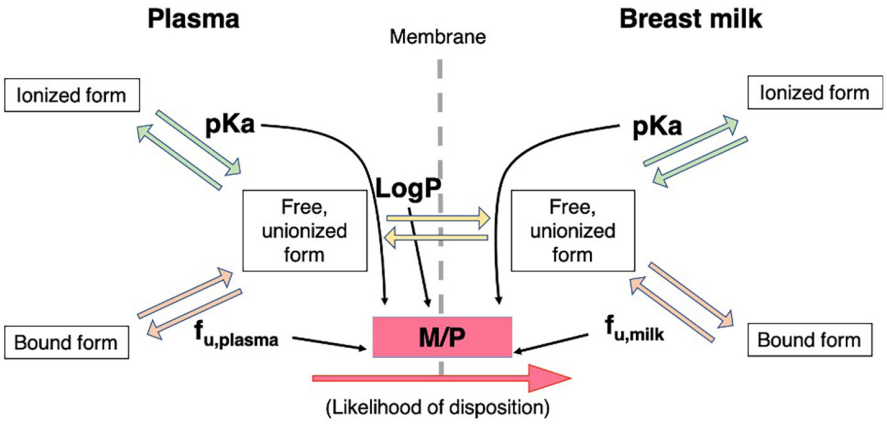

FIGURE 1 Schematic presentation of the theoretical concept. The likelihood of disposition of a small molecule to breast milk (*M*/*P*) will be predicted with physicochemical and pharmacokinetic parameters such as log*P*, pKa, $f_{u,\,plasma}$, and $f_{u,\,milk}$ where liphophilic, unionized, and unbound forms of the small molecule would distribute to milk.

TABLE 1 Predicted *M*/*P* values of amphoteric drugs

Drug name	Log*P*	Log$D_{7.2}$	$f_{u,p}$	pKa	*M*/*P* value		
					Observed	Previously reported[2a]	Corrected prediction[b]
Cefotaxime	−0.311	−4.16	0.64	2.66 (acidic) 2.90 (basic)	0.065[5,6]	0.012	0.012
Cefprozil	0.149	−2.18	0.60	2.92 (acidic) 6.93 (basic)	0.63[7]	0.012	0.046
Ceftriaxone	−1.76	−5.41	0.07	2.57 (acidic) 2.90 (basic)	0.04[8]	0.049	0.049
Cephapirin	0.792	−4.05	0.38	2.67 (acidic) 4.49 (basic)	0.13[5]	0.016	0.017
Ciprofloxacin	1.31	−0.831	0.60	2.74 (acidic) 8.76 (basic)	1.81[4]	0.009	0.823
Levodopa	−0.225	−1.80	0.64	2.24 (acidic) 9.30 (basic)	0.3[9]	0.012	0.874
Methotrexate	−0.276	−6.38	0.54	3.54 (acidic) 5.09 (basic)	0.15[10]	0.013	0.014
Ofloxacin	1.485	−0.17	0.75	2.27 (acidic) 6.81 (basic)	1.19[4]	0.006	0.031
Pefloxacin	2.164	0.20	0.80	2.75 (acidic) 7.03 (basic)	0.96[4]	0.001	0.046

Note: The drug name, log*P*, log$D_{7.2}$, $f_{u,p}$, pKa, and *M*/*P* values for the drugs of this study are listed. In this report, log$D_{7.2}$ values were obtained using the ChemAxon software.

[a]In the previous report,[2] log*P* values instead of log$D_{7.2}$ values were mistakenly used in Equation 5.

[b]In the present report, we used log$D_{7.2}$ values were used in Equation 5 as corrected and reported by Ilett and Hackett (2004) and Doogue et al., (2004).[11,12]

For Basic Drugs[1]:

$$\ln(M/P) = -0.09 + 2.54\ln(M_u/P_u) + 0.8\ln(f_{u,p}) + 0.46\ln K \quad (7)$$

For Acidic Drugs[1]:

$$\ln(M/P) = -0.405 + 9.4\ln(M_u/P_u) - 0.7\ln(f_{u,p}) - 1.5\ln K \quad (8)$$

If a molecule were neutral, which does not dissociate with ions, prediction of the *M*/*P* value uses the following equation (Equation 9), which was modified from a prior study[3]:

$$M/P = f_{u,p}/f_{u,m} \quad (9)$$

The set of prediction equations (Equation 1–8) can be used for amphoteric substances, which are chemicals that would act as an acid and as a base depending on pH. It is usual that amphoteric substances have multiple pKa values within a single chemical structure. Here, we need to understand that there are two types of amphoteric substances: Type 1 is a substance in which the acidic pKa is greater than the basic pKa, whereas Type 2 is a substance in which the basic pKa is greater than the acidic pKa. For Type 1 molecules, the substance can be classified as either an acid using the acidic pKa or a base using the

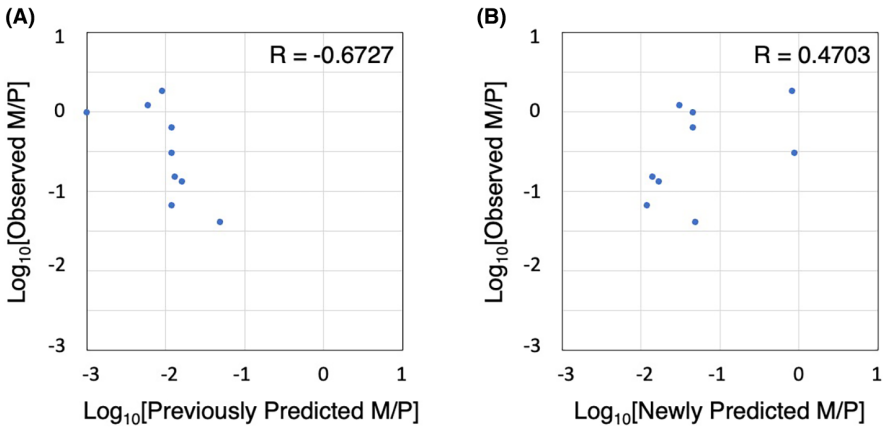

FIGURE 2 Graphic presentation of the correlation between observed and predicted *M/P* values. (A) Correlation between the observed and previously predicted *M/P* values was shown. (B) Correlation between the observed and currently predicted *M/P* values was shown. *R*, Pearson's correlation value.

basic pKa depending on which is closer to 7.2. For example, codeine is amphoteric with its acidic pKa of 13.42 and basic pKa of 8.19. This chemical will act as an acid and be negatively ionized at a pH greater than 13.42, whereas it will act as a base and be positively ionized at a pH lower than 8.19. Since its basic pKa is closer to 7.2, which is the pH of human breast milk, its basic pKa should be used in the equation and codeine should be treated as a base. In contrast, when dealing with a Type 2 compound where the acidic pKa is lower than the basic pKa, the situation necessitates a different approach, and the substance needs to be classified as either an acid using the basic pKa or a base using the acidic pKa depending on which is closer to 7.2. For example, ciprofloxacin has an acidic pKa of 2.74 and a basic pKa of 8.76. At pH between 2.74 and 8.76, this drug becomes a zwitterion, in which both acidic and basic functional groups are ionized and therefore it is electrically neutral. This drug will act as an acid and be negatively charged at a pH greater than 8.76, whereas it will act as a base and be positively charged at a pH lower than 2.74. Since its basic pKa is closer to 7.2, its basic pKa should be used in the equation and ciprofloxacin should be treated as an acid. The reason for this relates to one of the most basic interpretations of acids and bases: the Bronsted Lowry definitions. In this framework, acids are hydrogen donors whereas bases are hydrogen acceptors.

In the previous report, amphoteric drug ciprofloxacin was treated as an acid and its acidic pKa was used for the prediction of the *M/P* value.[2] Their prediction resulted in an *M/P* value of 0.01, which is a 180-fold underestimation as the observed *M/P* value for ciprofloxacin has been measured experimentally to be 1.81.[4] As discussed above, ciprofloxacin is a Type 2 amphoteric substance; therefore, this drug must be treated as an acid and its *basic* pKa must b used in prediction equations. When the basic pKa was used, the *M/P* value of ciprofloxacin was predicted to be 0.823, which is only approximately a two-fold underestimation. Cefotaxime, cefprozil, ceftriaxone, cephapirin, levodopa, methotrexate, ofloxacin, and pefloxacin are also amphoteric drugs that their proper pKa values were not used for prediction in the previous report.[2] Newly predicted *M/P* values of these amphoteric drugs were closer to the observed *M/P* values than as reported previously (Table 1). The previous prediction had an *R*-value of −0.6727; however, with the proper application of the equations and pKa values, our new prediction resulted in an *R*-value of 0.4703 (Figure 2).

The disposition of molecules from/to maternal plasma to/from milk can depend on the physicochemical properties of small molecules. However, in the mammary gland, there are transporter proteins potentially controlling the disposition of their substrates between plasma and milk. Since the prediction model (Equation 1–9) solely depends on the physicochemical properties of small molecules, naturally predicted *M/P* values would not be accurate for small molecules that are substrates for such transporters. This might explain the discrepancy between observed and predicted *M/P* values of certain drugs in Table 1 and Figure 2.

AUTHOR CONTRIBUTIONS

Sabrina Jones and Fatimah Al-Doori contributed to the acquisition of data. Sabrina Jones, Fatimah Al-Doori, and Ryoichi Fujiwara contributed to the analysis and interpretation of data. Sabrina Jones, Fatimah Al-Doori, and Ryoichi Fujiwara were involved in drafting the manuscript and revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ETHICS STATEMENT

This work does not involve human subjects or animals.

CONFLICTS OF INTEREST

We have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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