

Protein Binding Predictions in Infants

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ABSTRACT Plasma binding protein levels are lower in the newborn than in the adult and gradually increase with age. At birth, human serum albumin (HSA) concentrations are close to adult levels (75%-80%), while alpha 1-acid glycoprotein (AAG) is initially half the adult concentration. As a result, the extent of drug binding to HSA is closer to that of the adult than are those drugs bound largely to AAG. A model that incorporates the fraction unbound in adults and the ratio of the binding protein concentration between infants and adults successfully predicted the fraction unbound in infants and children.

KEYWORDS: plasma protein binding, fraction unbound, infants, children, newborn, albumin, alpha 1-acid glycoprotein.

INTRODUCTION

Dramatic and rapid improvements in drug elimination efficiency occur during the first months of postnatal development^{1, 2}. Yet few clinical pediatric studies have assessed changes in pharmacokinetics during infancy. This lack of specific clinical pharmacokinetic data for the infant population has confounded the design of drug dosage regimens and the assessment of risk associated with drug exposures in infants. A recent review proposes a tentative method to prospectively predict the contribution of hepatic P450-mediated metabolism and renal clearance due to glomerular filtration to infant systemic clearance at any age during the first 6 months following birth².

In addition to modeling the ontogeny of clearance pathways themselves, pharmacokinetic theory involves considering the extent of drug protein binding to fully appreciate the impact of changes in intrinsic clearance. The current work develops a tentative model to predict the fraction unbound of a specific drug from knowledge of the fraction unbound in adult serum. An estimation of the extent of drug binding to plasma proteins may improve the prediction of drug elimination capacity during infant development.

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HSA is the most abundant protein in plasma and interstitial fluid. HSA binds a number of relatively insoluble endogenous compounds, such as unesterified fatty acids, bilirubin, and bile acids, as well as a wide variety of drugs³. HSA has 2 distinct sites (I and II) that interact with drugs. Structural studies indicate that fatty acid binding sites are distributed throughout the protein, whereas most drugs bind to 1 of the 2 primary binding sites on the protein³. Drug site I, the so-called warfarin site, binds bilirubin, phenytoin, and warfarin, to name a few. Site II (the diazepam site) binds benzodiazepines, probenecid, and ibuprofen.

AAG is an acute phase reactant protein that contributes to the binding of a number of drugs, predominantly lipophilic cations⁴. There is considerable inter- and inpatient variability in AAG concentration in response to various diseases, trauma, or chemical insult⁵. AAG exists as a mixture of 2 or 3 genetic variants in the plasma of most individuals^{8, 9}. Many drugs have similar ligand binding constants, but other drugs (eg, promethazine, propafenone, amitriptyline, imipramine, warfarin, dipyridamole) demonstrated considerable differences between the 2 variants^{10, 11}.

THEORETICAL FRAMEWORK

Hepatic Clearance

The well-stirred model of hepatic clearance provides a simple model to describe the relationship between the physiological determinants that govern hepatic clearance (1):

$$Cl_H = \frac{Q_H f_u Cl_I}{Q_H + f_u Cl_I} \quad (1)$$

where Q_H represents the hepatic blood flow, f_u is the fraction unbound in plasma, and Cl_I is intrinsic clearance^{12, 13}. These physiological determinants exhibit individual rates and patterns of maturation during infant development. The previous paper² focused on modeling the development of enzyme activity as the primary determinant of hepatic clearance in infants. The current paper examines the ability to predict the fraction unbound in infants and children from adult values.

Protein Binding

The ratio of bound drug concentration (B) to free drug concentration (F) can be written in terms of the number

of binding sites (n), the molar protein concentration (P), and the affinity constant (K_A), as shown in Equation 2:

$$\frac{B}{F} = \frac{nPK_A}{1 + FK_A} \quad (2)$$

For most drugs in their therapeutic range, the product of F and K_A is much less than 1; therefore, the fraction unbound is drug concentration independent (ie, linear binding). Hence, Equation 2 could be written as Equation 3a and Equation 3b for the infant and adult, respectively.

$$\frac{B}{F_{infant}} = nP_{infant}K_{A,infant} \quad (3a)$$

$$\frac{B}{F_{adult}} = nP_{adult}K_{A,adult} \quad (3b)$$

If the intrinsic properties of the protein (n and K_A) are assumed to remain constant with age, Equation 3b may be rearranged to solve for a common K_A and subsequently substituted into Equation 3a. Such a rearrangement produces Equation 4, which predicts the B/F in infants in terms of the relative abundance of binding protein and the adult B/F .

$$\frac{B}{F_{infant}} = \frac{P_{infant}}{P_{adult}} \frac{B}{F_{adult}} \quad (4)$$

From a pharmacokinetics perspective, the fraction unbound is more valuable and more frequently reported than is B/F ratios. Fraction unbound drug in the plasma f_u is defined as the ratio of F to total drug concentration is the sum of F and B (equation 5).

$$f_u = \frac{F}{F + B} = \frac{1}{1 + \frac{B}{F}} \quad (5)$$

By substituting Equation 4 into 5, a relationship can be derived that predicts the fraction unbound for infants in terms of the ratio of the binding protein concentration (infant to adult) and the adult fraction unbound.

$$f_{u,infant} = \frac{1}{1 + \frac{P_{infant}}{P_{adult}} \frac{(1 - f_{u,adult})}{f_{u,adult}}} \quad (6)$$

As the ratio of abundance of binding proteins approaches unity, the fraction unbound in infants approaches adult values. The use of adult intrinsic clearance values may need to be adjusted for differences in binding for highly bound drugs in cases where the plasma protein concentration is significantly lower in infants.

Binding Protein Concentration Ratio

Numerous studies have examined the concentration of HSA and AAG in umbilical cord blood. The concentration of HSA is approximately 36 g/L in cord blood compared to 45 g/L in adult plasma¹⁴⁻²⁰. The infant-to-nonpregnant-adult concentration ratio for HSA is 0.81 (Table 1). In pregnant adults, HSA concentrations are lower throughout the later stages of pregnancy and appear to rebound to nonpregnant adult levels by 1 month postpartum^{14, 21, 22}, resulting in a ratio of 1.16 (Table 1). For the purposes of the predictive model, comparisons to nonpregnant adults are most relevant.

Table 1 - Serum Protein Concentration Ratios for Newborn Infants Compared to Maternal or Nonpregnant Adults for Alpha 1-Acid Glycoprotein (AAG) or Serum Albumin (HSA).

Protein	Adult	P_{infant}/P_{adult} (%)	References
AAG	Mother	38.0	(14, 22, 35)
	Nonpregnant adult	38.5	(14, 16-18, 23, 24)
HSA	Mother	116.3	(14, 22, 35, 36)
	Nonpregnant adult	81.1	(14, 16-20)

Cord AAG concentrations average 0.24 g/L compared to 0.60 g/L for normal adult plasma^{14, 16, 23, 24}, which results in an average ratio of 0.38 for AAG (Table 1). In contrast to HSA concentrations, AAG concentrations in the mother at delivery are similar to those of nonpregnant adults (Table 1). Following delivery, AAG concentrations rise and peak around 1 week postpartum^{14, 22}. Some studies also show an increase in neonatal serum AAG in the week following delivery¹⁴.

B/F Prediction

Figure 1 illustrates the relationship between age and infant-to-adult protein concentration ratio for the 2 major binding proteins from birth through adolescence (HSA: $r^2 = 0.400$; AAG: $r^2 = 0.460$). The inter- and intraindividual variation appears more pronounced for AAG than for HSA at all ages (Figure 1).

Figure 2 depicts the relationship between the B/F in cord (infant) plasma as a function of the B/F of the same drug in adult serum. For those drugs bound primarily to HSA a good correlation exists, while the relationship for drugs bound to AAG deviates considerably from unity. Fitting Equation 4 to the HSA data yields a high correlation coefficient ($r^2 = 0.970$) and an estimate of P_{infant}/P_{adult} of 0.592, which approximates the ratio described earlier (Table 1). The 95% confidence interval (0.535-0.660) does not include unity; hence, incorporating the binding protein concentration ratio proves to be a better model or

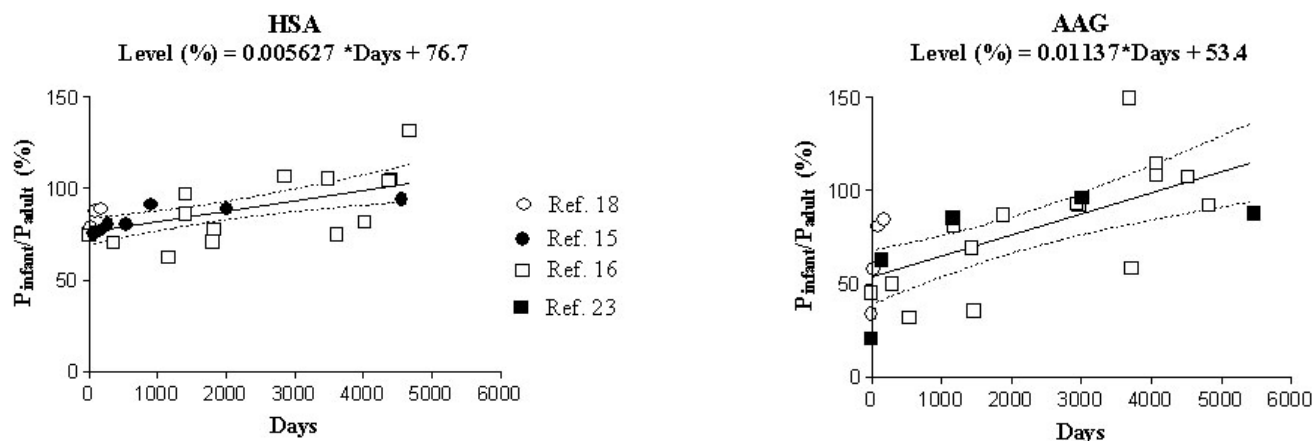


Figure 1 – Ratio of protein concentration in newborn or child relative to adult serum concentrations for serum albumin (HAS, left frame) and alpha 1-acid glycoprotein (AAG, right frame).

predicting B/F in infants than relying on the adult B/F alone.

The data are limited and more varied for AAG binding. The fit of Equation 4 to the AAG data yields a modest correlation coefficient ($r^2 = 0.713$) and an estimate of P_{infan} described earlier for AAG (Table 1). The 95% confidence interval (0.140-0.398) does not incorporate unity, again indicating that Equation 4 is a superior model for predicting B/F in infants. However, Figure 2 clearly shows that the relationship is less robust for AAG than for HSA because of several factors. First, the interindividual variation in AAG concentrations is considerably larger for AAG than for HSA. Second, all of the binding is ascribed to 1 protein in Equation 4. Most of the AAG-bound drugs are also bound to HSA. Combined with the limited number of observations for AAG, these factors contribute to the uncertainty of the prediction.

Fraction Unbound Prediction

Since protein binding data are more frequently presented and more useful clinically, Table 2 contains the observed and predicted fraction unbound as a function of age and binding protein for infants up to 1 year of age. This approach uses Equation 6 and requires estimates of the predicted binding protein concentration ratio (Figure 1) for the respective binding protein and age, as well as the adult fraction unbound, to predict the infant fraction unbound. Overall, the observations with respect to infants hold for plasma obtained from infants of any age. The model incorporating the binding protein ratio appears to more closely mimic the observed fraction unbound in infants than does the fraction unbound in adults alone. Moreover, the fraction unbound for HSA-bound drugs was more readily predicted than that for AAG.

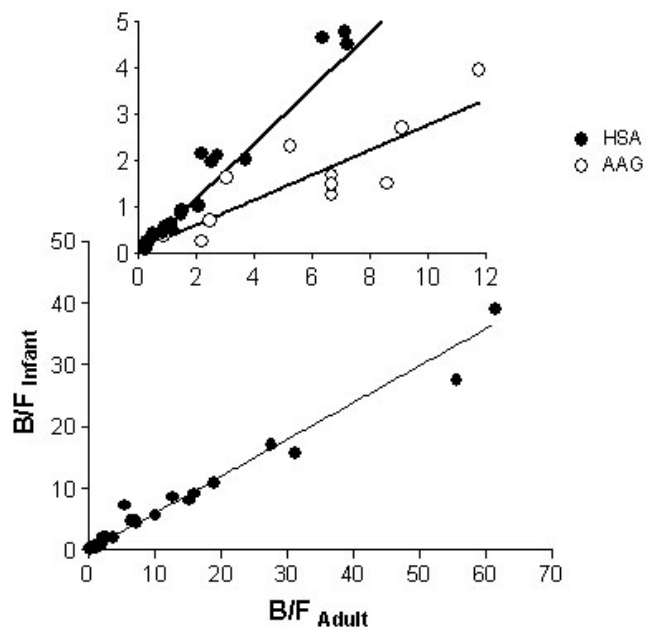


Figure 2 - B/F in infants (cord plasma) for a variety of drugs as a function of the adult B/F for drugs bound to HSA (closed circles). The lines represent weighted regression analysis of Equation 4 to the data. The inset represents an enlarged figure around the origin.

Table 2- Observed and Predicted (Equation Serum Protein Binding Free Fraction (f_u) Values for a number of Drugs Bound Predominantly to Alpha 1-Acid Glycoprotein (AAG) or Human Albumin (HSA), as a Function of Infant Age.

Age	Major Binding Protein (% Adult)	Drug Name	Child			Adult			
			f_u Observed	f_u Predicted	References	f_u	References		
Premature: 1 day	AAG (53.4)	Alfentanil	0.350	0.171	(37)	0.099	(38, 39)		
		Fentanyl	0.230	0.263	(37)	0.160	(40)		
	HSA (76.4)	Salicylic acid	0.175	0.154	(36)	0.123	(19, 25, 36, 41)		
Term: 1 day	AAG (53.4)	Alfentanil	0.269	0.171	(37, 38)	0.099	(38, 39)		
		Alprenolol	0.370	0.219	(14)	0.130	(14)		
		Desipramine	0.377	0.382	(19, 25)	0.248	(19, 25, 42)		
		Disopyramide	0.790	0.463	(30)	0.315	(30, 43)		
		Fentanyl	0.300	0.263	(37)	0.160	(40)		
		Lidocaine	0.585	0.433	(23, 24)	0.290	(23, 24, 44, 45)		
		Naloxone	0.715	0.687	(16)	0.540	(16, 46)		
		Propranolol	0.418	0.219	(24, 35)	0.130	(24, 47, 48)		
		Quinidine	0.400	0.219	(49)	0.130	(49, 50)		
		Sufentanil	0.201	0.137	(38, 51)	0.078	(38, 51, 52)		
		Verapamil	0.396	0.179	(35)	0.104	(35, 53)		
			HSA (76.4)	Ampicillin	0.900	0.848	(26)	0.810	(26, 54)
		Atropine		0.789	0.785	(19)	0.737	(19, 55)	
		Carbamazepine		0.330	0.262	(22, 56)	0.213	(22, 56, 57)	
		Chloramphenicol		0.541	0.471	(19)	0.405	(19, 58)	
		Chlordiazepoxide		0.060	0.040	(19)	0.031	(19, 59)	
		Cisplatin		0.150	0.115	(60)	0.090	(60)	
		Clonazepam		0.173	0.155	(31)	0.123	(31, 61)	
		Cloxacillin		0.110	0.080	(14)	0.062	(14, 62)	
		Diazepam		0.035	0.023	(24)	0.018	(24, 27, 63)	
		Digitoxin		0.084	0.064	(19)	0.050	(19, 64)	
		Digoxin		0.791	0.814	(65)	0.770	(64, 65)	
		Furosemide		0.025	0.021	(29)	0.016	(29, 66)	
		Meticillin		0.335	0.341	(19, 25)	0.284	(19, 25)	
		Morphine		0.690	0.727	(19)	0.670	(19, 67)	
		Nitrofurantoin		0.316	0.375	(19, 25)	0.314	(19, 25, 68)	
		Oxyphenbutazone		0.104	0.093	(19)	0.073	(19)	
Para-aminosalicylic acid	0.491	0.388		(19)	0.326	(19)			
Paracetamol	0.632	0.591		(19)	0.525	(19)			
Penicillin G	0.520	0.466		(26)	0.400	(26)			
Phenacetin	0.610	0.541		(19)	0.474	(19)			
Phenobarbital	0.658	0.528		(19, 26)	0.461	(19, 26, 69)			
Phenytoin	0.181	0.153		(19, 22, 26, 70)	0.122	(19, 22, 26, 70, 71)			
Promethazine	0.321	0.323		(19, 25)	0.268	(19, 25)			
Salicylic acid	0.056	0.045		(19, 25)	0.035	(19, 25)			
Sulfamethoxydiazine	0.099	0.075		(19, 25)	0.059	(19, 25)			

Age	Major Binding Protein (% Adult)	Drug Name	Child			Adult	
			f_u Observed	f_u Predicted	References	f_u	References
		Thiopental	0.177	0.171	(19, 20, 25)	0.136	(19, 20, 25, 72, 73)
		Tubocurarine	0.690	0.611	(24)	0.545	(24, 74)
		Valproate	0.121	0.191	(22, 75, 76)	0.153	(22, 76, 77)
Term: 1-7 days	AAG (53.4)	Alprenolol	0.360	0.219	(14)	0.130	(14)
		Cloxacillin	0.140	0.080	(14)	0.062	(14, 62)
Term: 7-28 days	HSA (76.4)	Ceftriaxone	0.285	0.115	(78)	0.090	(78, 79)
3-12 months	AAG (54.9)	Lidocaine	0.320	0.426	(23)	0.290	(23, 24, 44, 45)
		Quinidine	0.220	0.214	(49)	0.130	(49, 50)
		Sufentanil	0.115	0.141	(51)	0.083	(38, 51, 52)
	HSA (77.2)	Ceftriaxone	0.160	0.114	(78)	0.090	(78, 79)
		Phenytoin	0.147	0.152	(70)	0.122	(19, 22, 26, 70, 71)
		Valproate	0.143	0.107	(75, 80)	0.085	(22, 76, 77)

DISCUSSION

Rapid improvements in drug clearance occur during the first months of postnatal development¹. However, our understanding of the influence of clearance pathway ontogenesis and drug elimination efficiency in infants remains incomplete because of a lack of specific clinical pharmacokinetic data. We have used in vitro CYP450 activity data in fetal and infant livers and in vivo probe substrates of renal function to develop a general but tentative mathematical model that describes the ontogeny of individual clearance pathways in infants. Although invaluable, this approach is limited without a clear understanding of the ontogenesis of plasma protein binding in this population as well.

The model proposed herein is simple and straightforward. In the absence of direct measurements of plasma protein binding, the fraction unbound in an infant can be predicted from knowledge of the binding characteristics of the drug in adults and the known ontogeny for the binding protein (Figure 1). Clearly, there is less variability and there are better model predictions for a drug bound mainly to HSA. The enhanced variability associated with AAG is not unexpected given that it is an acute phase reactive protein subject to larger variability than HSA.

It should be noted that, in addition to lower HSA concentration, there is a considerable body of work that suggests that bilirubin and free fatty acids may influence the extent of binding of some drugs in the newborn^{14, 20, 22, 25}. For example, Nau et al reported that the elevations in free fatty acids shortly after birth result in increased free fractions of diazepam and its main metabolite²⁷. Ehrnebo et al reported that hyperbilirubinemia reduced

the binding of a number of acidic drugs²⁶. Elevated free fatty acids and bilirubin may explain some of the intersubject variance for a given drug within a population of infants, but these factors do not appear to be a global factor in the present analysis. One of the main assumptions in the model is that the affinity constant is similar in infants and adults. As a first approximation, this assumption appears to be well founded for most drugs, as evidenced by Figure 2 and Table 2. Herngren et al reported similar K_A values for newborns and mothers for cloxacillin (bound mainly to HSA) and for alprenolol (AAG bound)¹⁴. Pacifici et al reported that K_A values for furosemide were very similar in cord and adult plasma²⁹. Echizen et al reported that alterations in disopyramide were a function of binding capacity (ie, protein concentration) rather than affinity³⁰. By contrast, other groups have reported lower infant K_A values for clonazepam³¹, ceftriaxone³², and cefonicid and cefuroxime³³. Brodersen and Honore reported lower binding constants for the warfarin-binding site on isolated infant HSA, relative to HSA isolated from adult serum³⁴. These investigators reported similar binding constants for the diazepam-binding site in infant compared to adult HSA³⁴.

In the absence of direct infant measurements, Equation 6 provides a simple model to predict the fraction unbound in infant serum. The model appears to work well for a variety of drugs exhibiting an extensive range of binding.

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