British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2017) **83** 2595–2598 2595

EDITORIAL

Free dug concentrations in pregnancy: Bound to measure unbound?

Correspondence Stein Schalkwijk, PharmD, Department of Pharmacy, Radboud University Nijmegen Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands. Tel.: +3 124 361 6405; Fax: +3 124 366 8755; E-mail: stein.j.schalkwijk@radboudumc.nl

Received 14 June 2017; **Revised** 11 August 2017; **Accepted** 26 August 2017

Stein Schalkwijk[1,2], Rick Greupink[2] and David Burger[1]

[1]*Department of Pharmacy, Radboud Institute for Health Sciences (RIHS), Radboud University Medical Center, Nijmegen, The Netherlands and* [2]*Department of Pharmacology & Toxicology, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center, Nijmegen, The Netherlands*

Keywords albumin, antiretrovirals, pharmacokinetics, pregnancy, protein binding

> *"…the interpretation of total drug concentrations is complicated by the fact that pregnancy decreases albumin binding, which is expected to translate into lower total drug concentrations despite mostly unaffected unbound concentrations (pharmacologically active)."*

With this statement Marzolini *et al*. [1] underline a phenomenon of major importance when interpreting drug exposure during pregnancy. In the case presented, the authors find subtherapeutic total and free (unbound) elvitegravir concentrations during pregnancy. This is an important finding because subtherapeutic exposure of this antiretroviral drug puts women at an increased risk of virological failure and/or development of antiretroviral resistance, as well as an increased risk of perinatal HIV transmission during pregnancy [2, 3]. Unexpectedly, the authors also observe an increased protein binding during pregnancy. The statement above, combined with the findings of this case report, highlight that we cannot always rely on total concentrations for examining exposure during pregnancy. Here, we will first elaborate on the pharmacological mechanisms and physiology underlying the alterations in free and total concentrations during pregnancy. Next, we will present an overview of total and free concentrations for several other antiretroviral drugs during pregnancy. Finally, we will make some inferences based on the theory and the examples presented, and propose a rule of thumb for conducting clinical pharmacokinetic studies in pregnancy.

As mentioned in the opening statement, an important and well-described pharmacological process that changes during pregnancy is drug protein binding [4, 5]. The main binding proteins in plasma are albumin and α_1-acid glycoprotein (AAG) [6]. The concentrations of both are known to decrease during pregnancy [7]. When a drug binds to plasma proteins, changes in the protein concentrations may affect the fraction of the drug bound to proteins. The drug fraction that is not bound to protein is referred to as the fraction unbound (f_u). The f_u is defined as the free concentration (C_{free}) divided by the total concentration ($C_{tot} = C_{free} + C_{bound}$), equation (1).

$$f_u = \frac{C_{free}}{(C_{free} + C_{bound})} \quad (1)$$

Although this equation may be illustrative when calculating the f_u after measuring free and bound drug concentrations in plasma, it does not provide any information on how these parameters relate to each other from a mechanistic point of view. The f_u is controlled by the maximal protein binding capacity (B_{max}; proportional to the concentration of the binding protein, assuming one or more binding sites per protein with similar and constant binding affinity), the equilibrium dissociation constant (K_d; reflective of the binding affinity of the drug to the protein), and the free concentration of the drug, C_{free} (equation (2)).

DOI:10.1111/bcp.13432

$$f_u = \frac{K_d + C_{free}}{(B_{max} + K_d + C_{free})} \quad (2)$$

When C_{free} is much lower than K_d, which is usually the case, f_u depends on B_{max} and K_d only, according to equation (3) (adapted from [6]), and hence remains constant (*i.e.* linear binding).

$$f_u = \frac{K_d}{(B_{max} + K_d)} \quad (3)$$

Consequently, when protein concentrations decrease during pregnancy, the maximal protein binding capacity, B_{max}, decreases and hence f_u increases. During pregnancy K_d is expected to remain unchanged. Again, f_u does not depend on C_{free} nor C_{tot} (in case of linear binding). Instead, C_{free} and C_{tot} depend on the mechanistic pharmacological parameters K_d and B_{max} that define f_u.

Under steady-state conditions, the impact of f_u on the C_{tot} and C_{free} depends on whether drug clearance is restricted by protein binding [8]. When the rate of dissociation between drug and plasma protein is low compared to (intrinsic) elimination rate constants exhibited by the eliminating organs, total drug clearance will be restricted by protein binding. Note that intrinsic clearance is the intrinsic capacity of an eliminating organ to eliminate a drug and that the elimination rate constants characterizing the clearance processes are determined by physiology (e.g. enzyme and/or transporter abundance) and the affinity of the drug towards the metabolizing enzyme/transporter. When the dissociation rate of a drug from plasma proteins is high compared to the intrinsic clearance, total drug clearance will not be restricted by protein binding. In general, when drug clearance is restricted by protein binding, changes in f_u will affect C_{tot} but not C_{free}. When drug clearance is not restricted by protein binding, changes in f_u will affect C_{free} but not C_{tot} [9]. Since the clearance of most drugs that are highly bound to plasma proteins is restricted by protein binding, we will only discuss this scenario. Nevertheless, it should be noted that in some cases (e.g. with alterations in intrinsic clearance during pregnancy) clearance may theoretically change from being restricted to not being restricted by protein binding, or *vice versa*.

For drugs with protein binding-restricted clearance, steady-state C_{tot} is inversely proportional to f_u, and C_{free} is independent of f_u, whatever the mechanisms of elimination are [9].

$$C_{tot} = \frac{C_{free}}{f_u} \quad (4)$$

Although equation (4) looks like a trivial rearrangement of equation (1), it actually represents a more physiological relationship between f_u, C_{tot} and C_{free}. Here, C_{free} is the independent variable and f_u is the parameter determined by the mechanistic parameters K_d and B_{max}. C_{tot} is the dependent variable. When f_u changes, this relationship can be used to investigate the impact on C_{tot}, given the independent variable C_{free}. This leads to an important implication: decreases in protein concentrations during pregnancy can decrease C_{tot}, but not C_{free} and hence the pharmacological effect remains unchanged (see opening quote). Note that the latter is related to the notion that only unbound drug is available to equilibrate across membranes towards the site of action.

The fact remains that C_{free} can be decreased during pregnancy, also illustrated in Table 1. This, however, is typically related to other physiological changes during pregnancy such as an increased intrinsic clearance. As stated by Marzolini *et al*. [1], the abundance of certain cytochrome P450 enzymes or UDP-glucuronosyltransferases may be induced by alterations in hormone levels (e.g. progesterone) during pregnancy, resulting in higher hepatic intrinsic clearance. For drugs with protein binding-restricted clearance, the eliminating organ is only able to eliminate C_{free}, as bound drug is not available to the organ for elimination. Consequently, an increased intrinsic clearance lowers C_{free}. Changes in C_{free} can also relate to other physiological changes during pregnancy. For example, decreased intestinal motility during pregnancy may alter drug absorption and hence change C_{free}. Furthermore, pregnancy-induced changes in drug distribution may alter C_{free} (note that following an immediate phase of redistribution in plasma these changes also alter C_{tot}, the dependent variable).

To further illustrate this, Table 1 provides an overview of changes in free and total concentrations for several antiretroviral drugs measured in clinical pharmacokinetic studies during third trimester of pregnancy and *postpartum*. Typically, in these studies the *postpartum* pharmacokinetics serve as a control representing the nonpregnant situation as pregnancy-induced physiological processes are expected to have normalized 4–6 weeks *postpartum*. [15] All of these antiretroviral drugs are bound to plasma proteins and for most of them both C_{tot} and C_{free} decreased. For rilpivirine, the decreases in C_{tot} and C_{free} are rather similar, whereas for darunavir and lopinavir, the decrease in C_{tot} seems somewhat larger than the decrease in C_{free}. In all these cases fraction unbound was increased during pregnancy. Based on the theoretical considerations provided above, these results can be easily explained. Where the observed decreases in C_{free} are likely to be related to enzyme induction during pregnancy (or other factors that can affect C_{free}), the decreases in C_{tot} are most likely to be a result of the increased fraction unbound alongside enzyme induction. Differences between various drugs in the extent of the decrease in C_{tot} and C_{free} can have multiple reasons, including the fact that their pharmacokinetics rely on different physiological processes (e.g. binding to albumin *vs*. AAG) or differences in enzyme or protein affinity. Interestingly, etravirine C_{tot} was increased during pregnancy whereas C_{free} remained unchanged. Correspondingly, f_u was decreased during pregnancy, something also observed in the case presented by Marzolini *et al*. [1]: a peculiar finding that is not easily explained [13]. It may be related to a shift in (apparent) binding affinity, possibly because of pregnancy-related changes in plasma composition (e.g. endogenous lipids or proteins), or increased levels of other binding proteins during pregnancy, as hypothesized by Marzolini *et al*. [1]. Whatever the case, this indicates that during pregnancy total concentrations are not always a good surrogate for free concentrations, but also that despite our mechanistic knowledge of the pharmacokinetics during pregnancy, it still remains challenging to make inferences on C_{free} based on C_{tot}.

Table 1

Changes in total and free antiretroviral concentration during third trimester of pregnancy compared to *postpartum* with the corresponding fraction unbound

	Mean ratio C_{tot} (%) TT/PP	Mean ratio C_{free} (%) TT/PP	F_u (%) TT	F_u (%) PP	Ref
Rilpivirine (n = 11)					[10]
C_{min}	58	64	0.28[b]	0.23[b]	
C_{max}	80	90			
Darunavir (twice daily; n = 5)					[11]
C_{12}	89	105[a]	12	10	
C_{max}	76	92[a]			
Darunavir (once daily; n = 8)					
C_{24}	59	67[a]			
C_{max}	78	94[a]			
Darunavir (once daily; n = 12)					[12]
C_{min}	50	62	23	18	
C_{max}	69	84			
Etravirine (n = 10)					[13]
C_{min}	111	92	0.075	0.083	
C_{max}	135	107			
Lopinavir (n = 30)					[14]
C_{min}	69	79	0.84	0.68	

TT, third trimester; PP, *postpartum*; n, represents the number of paired observations
[a]Inferred from the reported fraction unbound during third trimester of pregnancy and postpartum.
[b]Inferred from reported secondary total and free PK parameters (C_{max}).

This leads us to the following rule of thumb: if we want to identify pregnancy-induced changes (or for any other condition in which protein concentrations are substantially altered) in the pharmacologically active C_{free}, for highly protein bound drugs we need to measure C_{free}. This is by no means a new insight [5, 16]. Still, for some clinical pharmacokinetic studies with highly bound drugs there is room for improvement as in general only C_{tot} is measured. This has a variety of reasons. The development of bioanalytical methods for measuring C_{free} can be challenging and validation is not straight-forward. [17] Also, measuring C_{free} is more costly in terms of material, time and personnel. It can also be argued that pregnancy-related decreases in C_{free} usually follow decreases in C_{tot}. Although this has been observed (most of the examples in Table 1), assuming this to be a reliable law is almost invariably wrong based on the considerations and examples presented. Overall, this underscores that as a community interested in optimizing pharmacotherapy during pregnancy, we need to push for measuring C_{free} when drugs are highly bound to plasma proteins.

Competing Interests

S.S., R.G. and D.B. declared no other relationships or activities that could appear to have influenced the submitted work.

We are grateful to Angela Colbers, Roeland Wasmann and Mette Benoist for their thorough revisions of the manuscript.

References

1 Marzolini C, Decosterd L, Winterfeld U, Tissot F, Francini K, Buclin T, *et al*. Free and total plasma concentrations of elvitegravir/cobicistat during pregnancy and postpartum: a case report. Br J Clin Pharmacol 2017; 83: 2835–8.

2 Harris NS, Fowler MG, Sansom SL, Ruffo N, Lampe MA. Use of enhanced perinatal human immunodeficiency virus surveillance methods to assess antiretroviral use and perinatal human immunodeficiency virus transmission in the United States, 1999–2001. Am J Obstet Gynecol 2007; 197 (3 Suppl): S33–41.

3 Townsend CL, Byrne L, Cortina-Borja M, Thorne C, de Ruiter A, Lyall H, *et al*. Earlier initiation of ART and further decline in mother-to-child HIV transmission rates, 2000-2011. AIDS 2014; 28: 1049–57.

4 Anderson GD. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. Clin Pharmacokinet 2005; 44: 989–1008.

5 Metsu D, Toutain PL, Chatelut E, Delobel P, Gandia P. Antiretroviral unbound concentration during pregnancy: piece of interest in the puzzle? J Antimicrob Chemother 2017; 72: 2407–9.

6 Trainor GL. The importance of plasma protein binding in drug discovery. Expert Opin Drug Discov 2007; 2: 51–64.

7 Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet 2012; 51: 365–96.

8 Toutain PL, Bousquet-Melou A. Free drug fraction vs free drug concentration: a matter of frequent confusion. J Vet Pharmacol Ther 2002; 25: 460–3.

9 Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and Applications, third edn. Philadelphia: Lippincott Williams & Wilkins, 1994.

10 Osiyemi O. Pharmacokinetics of Total and Unbound Rilpivirine in HIV-1–infected Pregnant Women. 2017.

11 Colbers A, Molto J, Ivanovic J, Kabeya K, Hawkins D, Gingelmaier A, *et al*. Pharmacokinetics of total and unbound darunavir in HIV-1-infected pregnant women. J Antimicrob Chemother 2015; 70: 534–42.

12 Crauwels H, Baugh B, Ryan A, Zorrilla C, Osiyemi O, Yasin S, *et al*. Total and unbound pharmacokinetics of once-daily darunavir/ritonavir in HIV-1-infected pregnant women. In: Abstracts of the 15th International Workshop on Clinical Pharmacology of HIV & Hepatitis Therapy, Washington, 2014. 2014(abstract O-15): p. 17–8.

13 Ramgopal M, Osiyemi O, Zorrilla C, Crauwels HM, Ryan R, Brown K, *et al*. Pharmacokinetics of total and unbound etravirine in HIV-1-infected pregnant women. J Acquir Immune Defic Syndr 2016; 73: 268–74.

14 Fayet-Mello A, Buclin T, Guignard N, Cruchon S, Cavassini M, Grawe C, *et al*. Free and total plasma levels of lopinavir during pregnancy, at delivery and *postpartum*: implications for dosage adjustments in pregnant women. Antivir Ther 2013; 18: 171–82.

15 Colbers A. Timing of the *postpartum* curve in pharmacokinetic studies in pregnancy should not be too early. 17th International Workshop on Clinical Pharmacology of HIV & Hepatitis Therapy; Washington DC2016.

16 Perucca E, Crema A. Plasma protein binding of drugs in pregnancy. Clin Pharmacokinet 1982; 7: 336–52.

17 Musteata FM. Monitoring free drug concentrations: challenges. Bioanalysis 2011; 3: 1753–68.