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COMMENTARY

Clinical Significance of the Plasma Protein Binding of Rifampicin in the Treatment of Tuberculosis Patients

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1 Introduction

The standard dose regimen for active pulmonary tuberculosis (TB) consists of an initial 2-month intensive treatment phase with rifampicin, isoniazid, pyrazinamide, and ethambutol, followed by a 4-month continuation phase with rifampicin, isoniazid, and ethambutol [1]. Despite the use of standard dose regimens, weight-banding, and directly observed treatment, the pharmacokinetics (PK) of rifampicin show very high interindividual variability, which may be explained by various factors, including variable oral absorption, pharmacogenetic differences in drug-metabolizing/transporter activities, nutritional status, sex differences, drug–drug interactions, comorbidities such as diabetes, and HIV co-infection [2–4]. The high interindividual variability in the PK of rifampicin leads to highly variable systemic exposure, with supratherapeutic plasma concentrations potentially leading to adverse reactions such as liver toxicity, and subtherapeutic plasma concentrations resulting in slow response to treatment and development of drug resistance [5, 6]. Consequently, therapeutic drug monitoring of first-line anti-TB drugs has been proposed to improve treatment outcomes in certain patient groups, such as slow responders, patients with diabetes, and those with HIV co-infection [7, 8]. This commentary focuses on the potential consequences of interpatient variability in plasma binding of rifampicin on its PK and pharmacodynamics (PD).

2 Plasma Protein Binding of Rifampicin: What is Known?

Earlier studies on the plasma binding of rifampicin reported an abnormally wide range of binding values (4–91%) when equilibrium dialysis was used, probably related to methodological problems [9–12]. In recent studies, the binding of rifampicin to plasma from TB patients receiving the four first-line anti-TB drugs, as measured by ultrafiltration and taking nonspecific binding to the ultrafiltration device into account, was very similar and ranged from approximately 72 to 92% [13–15]. Unlike rifampicin, the other first-line anti-TB drugs display a much lower plasma protein binding, and the reported values, although very different between studies, do not exceed 40% [14]. At such low plasma binding, interpatient variability will have only limited consequences for the drug's PK and PD. Indeed, only for drugs with a relatively high plasma binding, such as rifampicin, can a realistic interpatient variability in binding lead to an important change in the fraction unbound (f_u), which could then significantly contribute to the variability in the drug's PK/PD (Table 1).

Surprisingly, some fundamental aspects of the plasma protein binding of rifampicin have not yet been resolved. For example, it is not clear to what extent the various protein fractions in plasma contribute to the binding of rifampicin. By using electrophoresis, a higher binding of rifampicin to the γ-globulin compared with the albumin fraction was demonstrated in plasma from TB patients [11, 16]. In another study, the degree of binding of rifampicin was similar in whole plasma compared with aqueous solutions at physiological concentrations of albumin and α_1-acid glycoprotein [12]. Using fluorescence spectroscopy, a well-established technique to study drug–protein interactions, it was shown that binding of rifampicin to α_1-acid glycoprotein was negligible at therapeutic rifampicin plasma concentrations, but increased at higher concentrations [17]. Therefore, it is still not clear whether only albumin or other plasma proteins significantly contribute to the binding of rifampicin. In addition, conflicting results have been published on the effect of increasing plasma concentrations of rifampicin on its plasma binding [9, 12–15, 17].

Albumin and α_1-acid glycoprotein are the most important drug-binding proteins in plasma [18]. α_1-Acid glycoprotein

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Table 1 The effect of interpatient variability in percentage plasma binding of isoniazid (low binding) and rifampicin (high binding) on the unbound fraction in plasma

Drug	Interpatient variability	Binding (%)	f_u	f_u (fold change)
Isoniazid	Patient 1	0	1.00	1.3
	Patient 2	34	0.76	
Rifampicin	Patient 1	72	0.28	3.1
	Patient 2	91	0.09	

Variability is based on the plasma binding extremes found in 22 TB patients being treated with the four first-line anti-TB drugs [14]

TB tuberculosis, f_u unbound fraction in plasma

is an acute-phase plasma protein that has a high affinity for basic drugs, but it may also bind to neutral lipophilic molecules and to acidic drugs [19]. Whereas albumin plasma concentrations may be low in TB patients due to malnutrition, α_1-acid glycoprotein plasma concentrations have been shown to be abnormally high in TB patients, and to decrease during treatment with first-line anti-TB drugs [20]. Depending on which protein fractions are important, the plasma binding of rifampicin may not be stable in TB patients during treatment with first-line anti-TB drugs, thus potentially contributing to the inter- and intrapatient variability of the PK and PD of rifampicin.

3 Variability in Plasma Protein Binding and Pharmacokinetics of Rifampicin

Rifampicin is primarily eliminated by metabolism, presumably in the liver [10, 11]. The main metabolite, 25-desacetylrifampicin, which has been shown to retain approximately 50–100% of the antimicrobial activity of rifampicin against clinical isolates of *Mycobacterium tuberculosis*, is predominantly eliminated via biliary excretion [21, 22]. Renal excretion of unchanged rifampicin is a relatively unimportant route of elimination; following single-dose administration, the urinary excretion of rifampicin plus 25-desacetylrifampicin is < 20%, and decreases following multiple daily dose administration due to enzyme induction [11, 23]. Moreover, renal clearance (CL_r) of rifampicin is only approximately 12% of the glomerular filtration rate, indicating that filtration at the glomerulus, which is restricted to the unbound fraction of rifampicin in plasma, is very likely the main mechanism of renal excretion [10]. In addition, rifampicin seems to have a relatively low hepatic extraction coefficient, even after chronic administration, when the enzymes involved in its metabolism are auto-induced [24]. For all drugs that are nearly completely absorbed into the gut wall following oral administration, and are partly eliminated by the liver, the unbound plasma fraction (f_u) and hepatic intrinsic clearance ($CL_{h,int}$) are the driving forces for the hepatic contribution (CL_h) to the drug's plasma clearance (CL) [25, 26]:

$$CL_h = f_u \cdot CL_{h,int}.$$

Consequently, it is reasonable to assume that rifampicin's overall plasma CL ($CL = CL_h + CL_r$), i.e. both the hepatic and renal component, will be directly related to the unbound fraction of rifampicin in plasma. The plasma CL of rifampicin will therefore be approximately three times higher in a patient having a rifampicin plasma binding of 70% ($f_u = 0.3$) compared with another patient with a plasma binding of 90% ($f_u = 0.1$) (Table 1). As a result, the former patient will have total plasma concentrations that will only be approximately one-third of those in the latter patient, whereas the unbound plasma concentrations will be similar in both patients [26]. In such a situation, PK/PD analysis based on total plasma concentrations of rifampicin will be clearly misleading (see below).

In a recent systematic review, it was shown that based on a total of 21 publications that assessed the effect of HIV co-infection on the PK of rifampicin, most patient groups, both HIV-negative and HIV-positive, did not achieve the generally accepted threshold maximum concentration (C_{max}) for rifampicin of 8 μg/ml [3, 27]. In these studies, factors such as sex, age, severity of HIV infection (e.g. CD4 count), nutritional status and supplementation, diarrhea, antiretroviral treatment, and others were assessed for correlation with rifampicin systemic exposure measures (C_{max}, area under the plasma concentration–time curve [AUC]). Plasma protein binding of rifampicin was not measured in any of the studies. Serum albumin and α_1-acid glycoprotein levels were mentioned in only six and none of the 21 studies, respectively. In some studies, the serum albumin levels were normal in the patient groups investigated, whereas in others, some of the patients had hypoalbuminemia [28–31]. In two of the studies, rifampicin plasma levels (C_{max}, AUC) were substantially lower in patients with low serum albumin levels [32, 33]. The authors of this systematic literature review concluded that the heterogeneity in the systemic exposure to rifampicin remains largely unexplained. In our opinion, it is likely that the variability in plasma binding of rifampicin may have been a contributing factor that deserves more attention in future studies.

In an older study in undernourished TB patients with hypoalbuminemia on rifampicin therapy, the unbound plasma fraction of rifampicin ($f_u = 0.57$) was 2.6-fold higher and the AUC was 2.5-fold lower than in well-nourished control subjects ($f_u = 0.22$) [34]. The urinary recovery of rifampicin was also significantly reduced in undernourished patients, but it was not mentioned by how much. The reduced plasma protein binding of rifampicin and/or the

decrease in the oral absorption of rifampicin could explain the significantly reduced total plasma concentrations.

4 Variability in Plasma Protein Binding and Pharmacodynamics of Rifampicin

The antimicrobial activity of rifampicin is concentration-dependent and AUC/minimum inhibitory concentration (MIC) and C_{max}/MIC are the PK/PD indices closely related to long-term clinical outcome and the suppression of resistance, respectively [35, 36]. Since only the unbound drug is pharmacologically active, these indices should ideally be expressed in terms of unbound drug exposure, i.e. AUC_u/MIC and $C_{max,u}$/MIC [37]. In three recent studies, the unbound fraction of rifampicin in plasma of TB patients receiving the four first-line anti-TB drugs was found to vary approximately two to threefold [13–15]. In one of the studies, patients with TB were treated with either rifampicin 10 mg/kg or rifampicin 35 mg/kg combined with standard doses of isoniazid, pyrazinamide, and ethambutol [13]. AUC_u and $C_{max,u}$ of rifampicin showed a 3.6- and 6.4-fold variation, respectively, for the 10 mg/kg dose, and 2.4- and 1.9-fold variation for the 35 mg/kg dose. Because of this large interpatient variability in the systemic exposure to unbound rifampicin, it is important to express the target PD indices as AUC_u/MIC and $C_{max,u}$/MIC rather than AUC/MIC and C_{max}/MIC [37]. This concept was recently proposed to select the appropriate vancomycin dose in neonates in whom the unbound fraction of vancomycin (median 0.9) is 1.5-fold higher compared with that in adults (median 0.6) [38].

Finally, from a PK/PD standpoint, it is also important to consider the possible contribution of 25-desacetyl-rifampicin to the in vivo antimicrobial activity following treatment with rifampicin. The plasma concentrations of 25-desacetyl-rifampicin are much lower than those of rifampicin in patients treated with the four first-line anti-TB drugs. For example, Ruslami et al. found mean 25-desacetyl-rifampicin/rifampicin ratios for C_{max} and AUC of 0.16 and 0.13, respectively [39]. However, the microbially active part of the total plasma concentrations of 5-desacetyl-rifampicin and rifampicin is the fraction not bound to plasma proteins. No information was found in the literature regarding the plasma protein binding of 25-desacetyl-rifampicin. The plasma protein binding of a drug metabolite is usually lower than that of the parent compound, e.g. the plasma binding of the 25-desacetyl metabolite of rifapentine is 93%, compared with 98% for rifapentine [40]. Consequently, without any information on the plasma protein binding of 25-desacetyl-rifampicin, it is not possible to predict whether it is imperative to measure only rifampicin or rifampicin plus 25-desacetyl-rifampicin plasma levels for PK/PD analysis.

5 Conclusions

To unravel the contribution of plasma protein binding of rifampicin to interpatient variability in the PK and PD of this important first-line anti-TB drug, the following approach is proposed:

1. A standard, validated method should be used to measure the plasma protein binding of rifampicin in TB patients to make a comparison between the results obtained by different laboratories possible. Ultrafiltration may be the best choice because plasma binding of rifampicin based on equilibrium dialysis has shown extreme variability between studies.
2. In view of the fact that the antimicrobial activity of 25-desacetyl-rifampicin against clinical isolates of *M. tuberculosis* is similar to that of rifampicin, the plasma protein binding of this metabolite should be investigated to decide whether 25-desacetyl-rifampicin significantly contributes to the antimicrobial activity.
3. It is of utmost importance to establish the relative contribution of albumin, α_1-acid glycoprotein, and other plasma protein fractions such as lipoproteins to the plasma binding of rifampicin (and possibly 25-desacetyl-rifampicin).
4. When studying the PK of rifampicin, its unbound fraction in plasma should be measured, together with the serum levels of the proteins important for its binding, to prove (or disprove) the effect of plasma protein binding and fluctuations in their serum concentrations on the PK of rifampicin. The association between plasma protein concentrations (albumin, α_1-acid glycoprotein, etc.) and systemic rifampicin exposure (C_{max}, AUC) should be systematically explored.
5. In view of the large interpatient variability in the fraction of unbound rifampicin, the PK/PD target should be based on unbound rifampicin plasma concentrations (or possibly the unbound plasma concentrations of rifampicin + 25-desacetyl-rifampicin).

"I do not care about protein binding because I always measure free, unbound concentrations". Nicholas Holford (at the World Conference on Dosing of Antiinfectives: Celebrating the 150th Birthday of Paul Ehrlich, 9–11 September 2004, Nürnberg, Germany).

Compliance with Ethical Standards

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Conflict of interest Roger K. Verbeeck, Bonifasius S. Singu, and Dan Kibuule declare that they have no potential conflicts of interest that might be relevant to the contents of this manuscript.

References

1. World Health Organization. Treatment of tuberculosis guidelines, 4th ed. 2010. https://www.who.int/tb/publications/2010/9789241547833/en. Accessed 7 Dec 2018.
2. Devaleenal DB, Ramachandran G, Swaminathan S. The challenges of pharmacokinetic variability of first-line anti-TB drugs. Expert Rev Clin Pharmacol. 2017;10(1):47–58.
3. Daskapan A, Idrus LR, Postma MJ, Wilffert B, Kosterink JGW, Stienstra Y, et al. A systematic review of the effect of HIV infection on the pharmacokinetics of first-line tuberculosis drugs. Clin Pharmacokinet. 2019;58(6):747–66.
4. Stott KE, Pertinez H, Sturkenboom MGG, Boeree MJ, Aarnoutse R, Ramachandran G, et al. Pharmacokinetics of rifampicin in adult TB patients and healthy volunteers: a systematic review and meta-analysis. J Antimicrob Chemother. 2018;73(9):2305–13.
5. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis. 2013;208(9):1464–73.
6. Satyaraddi A, Velpandian T, Sharma SK, Vishnubhatla S, Sharma A, Sirohiwal A, et al. Correlation of plasma anti-tuberculosis drug levels with subsequent development of hepatotoxicity. Int J Tuberc Lung Dis. 2014;18(2):188–95.
7. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs. 2014;74(8):839–54.
8. Verbeeck RK, Günther G, Kibuule D, Hunter C, Rennie TW. Optimizing treatment outcome of first-line anti-tuberculosis drugs: the role of therapeutic drug monitoring. Eur J Clin Pharmacol. 2016;72(8):905–16.
9. Boman G, Ringberger VA. Binding of rifampicin by human plasma proteins. Eur J Clin Pharmacol. 1974;7(5):369–73.
10. Acocella G. Clinical pharmacokinetics of rifampicin. Clin Pharmacokinet. 1978;3(2):108–27.
11. Kenny MT, Strates B. Metabolism and pharmacokinetics of the antibiotic rifampin. Drug Metab Rev. 1981;12(1):159–218.
12. Woo J, Cheung W, Chan R, Chan HS, Cheng A, Chan K. In vitro protein binding characteristics of isoniazid, rifampicin, and pyrazinamide to whole plasma, albumin, and α-1-acid glycoprotein. Clin Biochem. 1996;29(2):175–7.
13. te Brake LHM, Ruslami R, Later-Nijland H, Mooren F, Teulen M, Apriani L, et al. Exposure to total and protein-unbound rifampin is not affected by malnutrition in Indonesian tuberculosis patients. Antimicrob Agents Chemother. 2015;59(6):3233–9.
14. Alghamdi WA, Al-Shaer MH, Peloquin CA. Protein binding of first-line antituberculosis drugs. Antimicrob Agents Chemother. 2018;61(7):e00641–718.
15. Litjens CHC, Aarnoutse RA, van Ewijk-Beneken Kolmer EWJ, Svensson EM, Colbers A, Burger DM, et al. Protein binding of rifampicin is not saturated when using high-dose rifampicin. J Antimicrob Chemother. 2019;74(4):986–90.
16. Buchanan N, Van Der Walt NA. The binding of antituberculous drugs to normal and Kwashiorkor serum. S Afr Med J. 1977;52(13):522–5.
17. Johnson DA, Smith KD. The efficacy of certain anti-tuberculosis drugs is affected by binding to α-1-acid glycoprotein. Biomed Chromatogr. 2006;20(6–7):551–60.
18. Bohnert T, Gan LS. Plasma protein binding: from discovery to development. J Pharm Sci. 2013;102(9):2953–94.
19. Smith SA, Waters NJ. Pharmacokinetic and pharmacodynamic considerations for drugs binding to alpha-1-acid glycoprotein. Pharm Res. 2018;36(2):30.
20. Almeida MLD, Barbieri MA, Gurgel RQ, Abdurrahman ST, Baba UA, Hart CA, et al. α1-Acid glycoprotein and α1-antitrypsin as early markers of treatment response in patients receiving the intensive phase of tuberculosis therapy. Trans R Soc Trop Hyg. 2009;103(6):575–80.
21. Dickinson JM, Aber VR, Allen BW, Ellard GA, Mitchison DA. Assay of rifampicin in serum. J Clin Pathol. 1974;27(2):457–62.
22. Furesz S. Chemical and biological properties of rifampicin. Antibiot Chemother. 1970;16:316–51.
23. Ellard GA, Fourie PB. Rifampicin bioavailability: a review of its pharmacology and the chemotherapeutic necessity for ensuring optimal absorption. Int J Tuberc Lung Dis. 1999;3(11):S301–8.
24. Loos U, Musch E, Jensen JC, Mikus G, Schwabe HK, Eichelbaum M. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. Klin Wochenschr. 1985;63(23):1205–11.
25. Wilkinson GR, Shand DG. A physiological approach to hepatic drug clearance. Clin Pharmacol Ther. 1975;18(4):377–90.
26. Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther. 2002;71(3):115–21.
27. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs. 2002;62(15):2169–83.
28. Antwi S, Yanh H, Enimil A, Sarfo AM, Gillani FS, Anong D, et al. Pharmacokinetics of the first-line antituberculosis drugs in Ghanaian children with tuberculosis with or without HIV coinfection. Antimicrob Agents Chemother. 2017;61(2):e01701–16.
29. Bekker A, Schaaf HS, Draper HR, van der Laan L, Murray S, Wiesner L, et al. Pharmacokineics of rifampin, isoniazid, pyrazinamide, and ethambutol in infants dosed according to revised WHO-recommended treatment guielines. Antimicrob Agents Chemother. 2016;60(4):2171–9.
30. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob Agents Chemother. 2006;50(4):1170–7.
31. Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, Wells CD, et al. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin Infect Dis. 2009;48(12):1685–94.
32. Ramachandran G, Kumar AK, Bhavani PK, Kannan T, Kumar SR, Gangadevi NP, et al. Pharmacokinetics of first-line antituberculosis drugs in HIV-infected children with tuberculosis treated with intermittent regimens in india. Antimicrob Agents Chemother. 2015;59(2):1162–7.
33. Ramachandran G, Kumar AK, Kannan T, Bhavani PK, Kumar SR, Gangadevi NP, et al. Low serum concentrations of rifampicin and pyrazinamide associated with poor treatment outcomes in children with tuberculosis related to HIV status. Pediatr Infect Dis J. 2016;35(5):530–4.
34. Polasa K, Murthy KJR, Krishnaswamy K. Rifampicin kinetics in undernutrition. Br J Clin Pharmacol. 1984;17(4):481–4.
35. Gumbo T, Louie A, Deziel MR, Liu W, Parson LM, Salfinger M, et al. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob Agents Chemother. 2007;51(11):3781–8.
36. Pasipanodya J, Gumbo T. An oracle: antituberculosis pharmacokinetics–pharmacodynamics, clinical correlation, and clinical trial simulations to predict the future. Antimicrob Agents Chemother. 2011;55(1):24–34.
37. Gonzalez D, Schmidt S, Derendorf H. Importance of relating efficacy measures to unbound drug concentrations for anti-infective agents. Clin Microbiol Rev. 2013;26(2):274–88.
38. Leroux S, van den Anker JN, Smits A, Pfister M, Allegaert K. Maturational changes in vancomycin protein binding affect vancomycin dosing in neonates. Br J Clin Pharmacol. 2019;85(5):865–7.

39. Ruslami R, Nijland HMJ, Adhiarta IGN, Kariadi SHKS, Alisjahbana B, Aernoutse RE, et al. Pharmacokinetics of antituberculosis drugs in pulmonary tuberculosis patients with type 2 diabetes. Antimicrob Agents Chemother. 2010;54(3):1068–74.

40. Egelund EF, Weiner M, Singh RP, Prihoda TJ, Gelfond JAL, Derendorf H, et al. Protein binding of rifapentine and its 25-desacetyl metabolite in patients with pulmonary tuberculosis. Antimicrob Agents Chemother. 2014;58(8):4904–10.