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Review

The inhibitory and inducing effects of ritonavir on hepatic and intestinal CYP3A and other drug-handling proteins

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ABSTRACT

Ritonavir, originally developed as HIV protease inhibitor, is widely used as a booster in several HIV pharmacotherapy regimens and more recently in Covid-19 treatment (e.g., Paxlovid). Its boosting capacity is due to the highly potent irreversible inhibition of the cytochrome P450 (CYP) 3 A enzyme, thereby enhancing the plasma exposure to coadministered drugs metabolized by CYP3A. Typically used booster doses of ritonavir are 100–200 mg once or twice daily. This review aims to address several aspects of this booster drug, including the possibility to use lower ritonavir doses, 20 mg for instance, resulting in partial CYP3A inactivation in patients. If complete CYP3A inhibition is not needed, lower ritonavir doses could be used, thereby reducing unwanted side effects. In this context, there are contradictory reports on the actual recovery time of CYP3A activity after ritonavir discontinuation, but probably this will take at least one day. In addition to ritonavir's CYP3A inhibitory effect, it can also induce and/or inhibit other CYP enzymes and drug transporters, albeit to a lesser extent. Although ritonavir thus exhibits gene induction capacities, with respect to CYP3A activity the inhibition capacity clearly predominates. Another potent CYP3A inhibitor, the ritonavir analog cobicistat, has been reported to lack the ability to induce enzyme and transporter genes. This might result in a more favorable drug-drug interaction profile compared to ritonavir, although the actual benefit appears to be limited. Indeed, ritonavir is still the clinically most used pharmacokinetic enhancer, indicating that its side effects are well manageable, even in chronic administration regimens.

1. Introduction

Ritonavir (Fig. 1), originally developed as an HIV protease inhibitor, is widely used as an oral booster (enhancing the plasma exposure to other drugs) in HIV pharmacotherapy regimens and more recently in Covid-19 treatment, such as with Paxlovid (a combination of nirmatrelvir and ritonavir) [1–6]. In HIV regimens, addition of ritonavir will partly prevent the development of resistance to the HIV therapy by making plasma exposure to the other drug(s) higher and more consistent [1,3]. Boosting also allows for less frequent dosing of the drug(s), potentially leading to improved patient adherence. The boosting capacity of ritonavir is caused by its efficient inhibition of the cytochrome P450 3A (CYP3A) enzyme, leading to reduced metabolism of concomitantly administered CYP3A substrate drugs [1,3].

CYP enzymes are an essential superfamily of monooxygenases, which are involved in the biotransformation of many endogenous and xenobiotic substances, including drugs [7,8]. The most important and best studied isoform of the CYP3A subfamily is CYP3A4, which is abundant in the liver and intestines, and can metabolize a structurally highly diverse set of compounds, encompassing some 50 % of the currently used drugs. Another important subfamily member is CYP3A5, which has extensive substrate overlap with CYP3A4 [7,9]. CYP3A5 is polymorphically expressed in the liver and intestine, and its expression level is highly dependent on the (ethnically varied) genotype of an

Abbreviations: AUC, area under the curve; BCRP/ABCG2, breast cancer resistance protein; b.i.d., twice daily; C_{max}, maximum plasma concentration; CYP, Cytochrome P450; HIV, human immunodeficiency virus; HLMs, human liver microsomes; IV, intravenous; MATE1, multidrug and toxin extrusion protein1; MRP2, multidrug resistance-associated protein 2; $OATP_s$/SLCO, organic anion transporting polypeptides; PBPK model, physiologically based pharmacokinetic model; P-gp/ABCB1, P-glycoprotein; PXR, pregnane X receptor; $t_{1/2}$, half-life of a drug; t.i.d., thrice daily; UGT, uridine diphosphate-glucuronosyl transferase.

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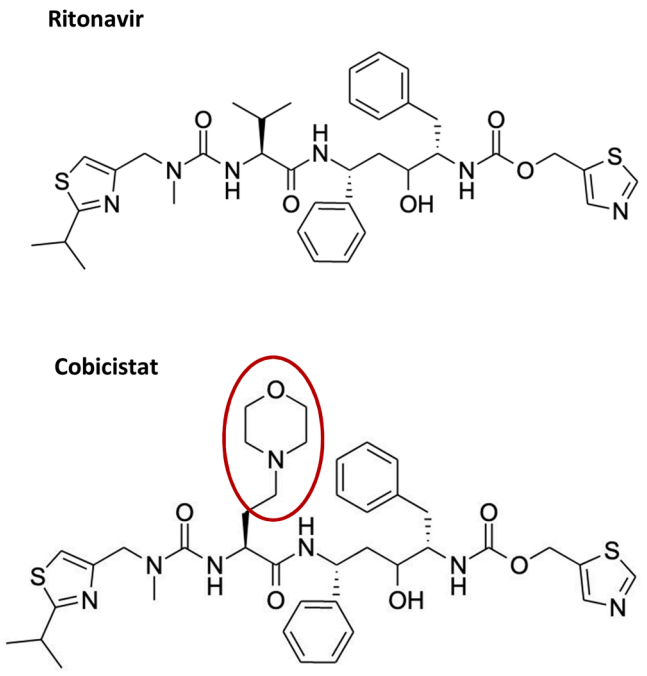

Fig. 1. : Chemical structures of ritonavir and cobicistat, illustrating their similarity. The only divergent side chain is highlighted in red in cobicistat.

individual [7]. Importantly, however, there is no obvious difference in inhibitory efficacy of ritonavir towards CYP3A4 and CYP3A5 [10,11]. The essentially irreversible inhibition (or inactivation) of CYP3A4/5 by ritonavir leads to enhancement of pharmacokinetic parameters of CYP3A substrate drugs, such as the plasma concentration area under the curve (AUC), maximum concentration (C_{max}) and half-life ($t_{1/2}$), resulting in increased bioavailability. Typically used booster doses of ritonavir in patients are 100–200 mg once or twice a day, because this guarantees nearly complete inhibition of the CYP3A enzyme [1,12]. These boosting doses appear to be well tolerated and have demonstrated good efficacy [1]. An overview of currently registered treatment regimens in which ritonavir is applied as a booster is shown in Table 1.

The pharmacokinetics of ritonavir in humans have been well studied. Ritonavir has a high plasma protein binding (around 98 %), and the time to reach the maximum plasma concentration after oral administration is approximately four hours [13,14]. It is itself primarily metabolized by CYP3A, and to a lesser extent by CYP2D6, to form at least four major metabolites [13–15]. Probably there is auto-induction of the metabolism of ritonavir, mainly by raising CYP3A levels, which will stabilize after two weeks, yielding a steady state [14]. Ritonavir elimination is primarily via feces as unchanged drug, perhaps illustrating that it effectively reduces also its own metabolism by CYP3A, and a minor fraction is eliminated by renal excretion [13,14].

It may be useful to clarify here why it is thought that ritonavir can be simultaneously a highly efficient, irreversible inhibitor of CYP3A, but can also be metabolized by it, which seems paradoxical. Ritonavir is a relatively complex, linear, and flexible molecule, with many rotatable bonds (Fig. 1). Also the catalytic drug binding site of CYP3A is known to be flexible and capable of "induced fit" structural changes depending on the substrate molecule it binds [10,11]. This combination of properties likely allows for multiple different configurations of initial binding of ritonavir to the CYP3A active site. The fate of ritonavir either ending up being metabolized by CYP3A or binding irreversibly and inactivating it, may therefore simply depend on the configuration and orientation in which ritonavir is initially bound in the CYP3A active site. Some initial configurations will result in chemical modification (metabolism) of ritonavir and subsequent full release of the ritonavir metabolites, whereas other configurations will lead to irreversible binding of either ritonavir itself or one or more of its metabolites to CYP3A [16]. As a freed-up CYP3A enzyme will again be able to bind new ritonavir molecules, each time with a certain chance of resulting in an irreversibly inactivated state, over time the fraction of metabolically competent CYP3A will decline. Alternatively, or, more likely, additionally, some ritonavir metabolites formed by CYP3A (such as *N*-ritonavir) can be highly reactive, and, upon release and subsequent rebinding to CYP3A, directly inactivate it. For a more detailed discussion of these aspects see Loos et al. [17].

The use of ritonavir as pharmacokinetic enhancer (booster) is mainly because of its potent and essentially irreversible inhibitory effects on CYP3A4/5. Additionally, ritonavir can also inhibit drug transporters to some extent, such as P-glycoprotein (P-gp/ABCB1), Breast Cancer Resistance Protein (BCRP/ABCG2) and organic anion-transporting polypeptides (OATPs/SLCO). Ritonavir exhibits to a lesser extent the ability to induce CYP2D6, CYP2C8, CYP2C9 and CYP219 enzymes [18]. This mixture of inhibitory and inducing effects of ritonavir on other enzymes and drug transporters might sometimes lead to undesirable drug-drug interactions during concomitant use with other drugs, when ritonavir is applied as a booster. For example, the dosage of metoprolol, a CYP2D6 substrate, may need to be adjusted when administration is concurrent with ritonavir [19]. Due to ritonavir's irreversible nature of CYP3A4/5 inhibition, it will not only increase the plasma levels of the intended boosted drugs, but it will also enhance the plasma exposure to other concomitantly used medications, if these are CYP3A substrates as well. This may require dose reductions of the other used medication. Therefore, it is essential to strive for the lowest effective boosting dose of

Table 1
Overview of current drug combinations with ritonavir acting as booster.

Drug indication	Drug name (brand name)	Dose	Dosage form	Ritonavir dose	Dosage form	Combination or separate formulation (s)	Company
HIV	Atazanavir (Reyataz)	300 mg once daily	Capsules	100 mg once daily	Tablets	Separate	Bristol-Myers Squibb
	Darunavir (Prezista)	800 mg once daily	Tablets / oral suspension	100 mg once daily	Tablets	Separate	Janssen Cilag
	Fosamprenavir (Telzir)	700 mg b.i.d.	Tablets	100 mg b.i.d.	Tablets	Separate	ViiV Healthcare
	Indinavir (Crixivan)	400 mg b.i.d.	Capsules	100 mg b.i.d.	Tablets	Separate	MSD
	Lopinavir (Kaletra)	400 mg b.i.d.	Tablets / oral solution	100 mg b.i.d.	NA	Combination formulation	AbbVie
	Saquinavir (Invirase)	500 mg b.i.d.	Tablets	100 mg b.i.d.	Tablets	Separate	Roche
	Tipranavir (Aptivus)	500 mg b.i.d.	Capsules	200 mg b.i.d.	Tablets	Separate	Boehringer Ingelheim
Hepatitis C	Paritaprevir + Ombitasvir (Viekirax)	150 mg + 25 mg once daily	Tablets	100 mg once daily	NA	Combination formulation	AbbVie
SARS-COVID-19	Nirmatrelvir (Paxlovid)	300 mg b.i.d. for 5 days	Tablets	100 mg b.i.d.	Tablets	Separate	Pfizer

b.i.d., twice daily

ritonavir allowing sufficiently high trough levels of the target drug.

Despite the wide use of ritonavir as pharmacokinetic enhancer, the exact mechanism of irreversible CYP3A inactivation is not fully understood, as we discussed in more detail in a recent review [17]. Nonetheless, there can be no question that ritonavir functions as an irreversible inhibitor of CYP3A. Nowadays ritonavir is not only used as a booster for HIV and more recently Covid-19 regimens, but it is also investigated as a booster for other drugs, for example oral taxanes. Taxanes have a low oral availability due to high first-pass metabolism by CYP3A4/5 in the liver and intestine. Therefore, coadministration of ritonavir with these drugs could enhance the oral availability of orally administered taxanes [20–22]. This is one example of a drug that can be boosted by ritonavir, but in the future, this may also be possible for other drugs. Additionally, for some currently running research programs, lower doses of ritonavir compared to the typically used boosting doses could also be of value, especially when combining ritonavir with (cyto-) toxic drugs [20,21,23]. These classes of drugs have a higher risk of (severe) side effects and this initial risk will increase when such drugs are boosted by ritonavir due to higher plasma exposure. This could be a reason to strive for the lowest suitable boosting dose.

From this perspective, we are interested in obtaining a better understanding of ritonavir itself and its impact on CYP3A. Therefore, the aim of this review is to summarize what is known from the literature based on clinical studies about the influence of ritonavir on hepatic and intestinal CYP3A activity, and on other drug-metabolizing enzymes and drug transporters. Furthermore, we compare ritonavir with other known strong CYP3A inhibitors, such as ketoconazole and cobicistat.

2. Time- and dose-dependency of the impact of ritonavir on CYP3A activity

An interesting question to study is whether ritonavir is able to boost the plasma concentration and consequently the tissue accumulation of a boosted drug after a single ritonavir administration compared to repeated administration. This will give more insights into the time- and dose-dependency of ritonavir in inhibiting the CYP3A enzyme. This could be of value for novel ritonavir boosting regimens, in which one aims to keep the boosting frequency and dose to a minimum, e.g. when combining ritonavir with (cyto-)toxic drugs. Additionally, this will prevent patients from unnecessary exposure to ritonavir and minimize the risk of extra side effects (owing to the combination with other toxic drugs) compared to the typically used boosting doses in HIV regimens, which by themselves are tolerated well (Table 1). Therefore, it could be interesting to study to what extent repeated dosing will lead to higher inactivation of the CYP3A enzyme. We therefore here also review literature about the observed impact on CYP3A after a single dose versus repeated doses of ritonavir in humans. An overview of the discussed studies is presented in Table 2. We reviewed different types of study designs; some of the studies were solely interested in the impact of a single-dose administration on the CYP3A enzyme, in contrast to other studies in which they compared single versus repeated ritonavir dosing.

The ritonavir dose- and concentration-dependency of CYP3A inhibition after a single dose of ritonavir was examined by Eichbaum et al. in healthy participants (n = 12) [12]. Midazolam (3 mg orally), a typical CYP3A substrate, was used as a probe drug to evaluate the CYP3A activity. They observed that after a single oral administration of ritonavir, the plasma concentrations and AUC of midazolam were enhanced in a ritonavir dose-dependent manner. The greatest increase was observed at

Table 2
Study designs of reviewed clinical studies of ritonavir as a booster.

Study	Number and characteristics of participants	Study design	Probe drug (s)	Ritonavir dose	Treatment period	Treatment group
Kharash et al. [24]	12 healthy non-smoking participants (male n = 6, female n = 6), age: 19–34 years	A 3-session sequential crossover design	Alfentanil (oral and IV) and fexofenadine (oral)	Day 1: 200 mg t.i.d. Day 2–7: 300 mg b.i.d. - Day 8–21: 400 mg b.i.d.	21 days	Every participant received the same treatments
Mathias et al. [25]	24 healthy non-smoking participants (male n = 15, female n = 9), age: 18–45 years (21 subjects evaluable)	A “leapfrog” study design	Elvitegravir (oral) and midazolam (IV)	A: 20 mg B: 50 mg C: 100 mg D: 200 mg Once daily oral for 10 days	21-day treatment phase, followed by 7-day follow-up phase	Each subject received two treatments consecutively: A and C, or B and D
Katzenmaier et al. [26]	16 healthy non-smoking participants (male n = 9, female n = 7), age: 18–50 years	Open, single-center, randomized design	Midazolam (oral)	300 mg b.i.d. (day 1–8) 300 mg once daily (day 9)	12 days	Two groups: one received voriconazole (day 1: 400 mg b.i.d; day 2–8: 200 mg b.i.d.; day 9: 200 mg) and the other ritonavir
Kirby et al. [27]	16 healthy non-smoking participants (male n = 5, female n = 11), age: 20–50 years	Staggered study design	A: midazolam (oral) and digoxin (oral) B: midazolam (IV), dextromethorphan (oral), tolbutamide (oral), and caffeine (oral)	Day 1: 200 mg t.i.d. Day 2–7: 300 mg b.i.d. >Day 8: 400 mg b.i.d.	14 days	Two arms: A: ritonavir or rifampin (600 mg once daily) B: nelfinavir (1250 mg b.i.d.) or rifampin (600 mg once daily)
Kirby et al. [28]	9 healthy non-smoking Caucasians (male n = 3, female n = 6), age: 18–42 years	Simultaneous study design	Midazolam (oral), digoxin (oral), and bupropion (oral)	Day 1: 200 mg t.i.d. Day 2–7: 300 mg b.i.d. >Day 8: 400 mg b.i.d.	15 days	All subjects treated with ritonavir, nelfinavir (1250 mg b.i.d.) or rifampin (600 mg once daily)
Eichbaum et al. [12]	12 healthy non-smoking Caucasians (male n = 8, female n = 4), age: 19–50 years	Randomized, open clinical trial with fixed sequence design	Midazolam (oral)	Rising doses of 0.1, 1, 10, 100 mg (group A) and 0.3, 3, 30 and 300 mg (group B)	4 days	Two dosing groups of 6 participants with four doses each

b.i.d., twice daily; t.i.d., thrice daily.

the highest dose of ritonavir, 300 mg, reducing the clearance of midazolam to ~10 % of baseline [12]. Eichbaum et al. found 81.4 % inhibition after a single dose of 100 mg ritonavir, as judged by (oral) midazolam clearance, so only slightly less than the 90 % clearance reduction found at the 300 mg dose [12]. Higher inhibition levels could likely be achieved with repeated 100 mg administrations (twice daily), which will increase the inhibitory effect of ritonavir. Since ritonavir is primarily a mechanism-based inhibitor, it may take some time to reach maximum inhibition levels. If full inhibition of CYP3A is not needed, for instance to assess whether clearance of a probe drug is likely mediated by CYP3A, then a lower dose of ritonavir can be used, for example 10 mg. Even after a single dose of 3 mg ritonavir in healthy participants, there was still a 50% reduction observed in CYP3A activity as measured by midazolam clearance [12].

3. Auto-inhibition by ritonavir

Interestingly, the study by Eichbaum et al. showed that ritonavir itself exhibits nonlinear pharmacokinetics when reducing the dose, resulting in an almost 900-fold reduction in the dose-corrected plasma exposure going from the highest dose, 300 mg, to the lowest, 0.1 mg of ritonavir. The authors suggested that this observed pharmacokinetic nonlinearity of ritonavir is likely due to so-called "auto-inhibition": ritonavir is able to inhibit its own metabolism through inactivating CYP3A, even after a single administration. This inhibition will be less extensive at lower ritonavir doses and therefore there will be comparatively higher ritonavir elimination and thus reduced ritonavir exposure [12]. It is worth noting that, given the formation of irreversible complexes between ritonavir and CYP3A, it could be that higher dosages of ritonavir irreversibly inactivate virtually all accessible CYP3A, whereas lower dosages can only inactivate a fraction of all available CYP3A.

This auto-inhibition hypothesis is supported by work from the group of Mathias et al. in healthy participants (n = 24). They also observed a far greater than proportional increase in plasma exposure to ritonavir with increasing ritonavir doses. In this case this concerned a steady-state, once-daily administration regimen of oral ritonavir together with a fixed oral dose of the probe drug elvitegravir (125 mg) [25]. Increasing the daily oral ritonavir dose from 20 to 200 mg increased the ritonavir plasma AUC by 119-fold, which was likewise interpreted as pharmacokinetic nonlinearity due to auto-inhibition of ritonavir metabolism. Furthermore, they observed a 66 % reduction of intravenous (IV) midazolam probe drug clearance after administration of 20 mg oral ritonavir (once daily). Overall in this study, the maximum inhibition in IV midazolam clearance that was reached was 82% and 83% after once daily 100 and 200 mg ritonavir, respectively [25]. It is worth noting that this probably mainly concerned inhibition of hepatic CYP3A, as intestinal CYP3A activity will likely hardly affect IV midazolam clearance.

4. Use of lower ritonavir boosting doses and their impact on CYP3A activity

Mathias et al. further suggested that lower doses than the usual boosting doses (100–200 mg, see Table 1) of ritonavir could be applied for pharmacokinetic enhancement of other drugs, since they already observed substantial reductions in CYP3A activity at a 20 mg dose [25]. Consistent with the observations of Eichbaum et al., the systemic ritonavir concentrations detected at a clearly CYP3A inhibitory dose of 20 mg were well (at least one order of magnitude) below the reported IC_{50} values for CYP3A4 in vitro [12,25]. These observations indicate that systemic plasma levels of oral ritonavir may not be the best parameter to assess the achieved level of CYP3A inhibition in humans. In contrast, estimates of the local gut and portal vein concentrations of ritonavir over 1 h after an oral 20 mg ritonavir dose were found to be, respectively, far above (gut), and at or well above (portal vein) the reported in vitro IC_{50} values, in accordance with the experimentally observed levels of inhibition of (first-pass) CYP3A activity [25]. More recently, Umehara et al. (2018) designed a physiologically based pharmacokinetic (PBPK) model using four different oral ritonavir doses, respectively 20, 50, 100 and 200 mg once daily over at least 10 or 11 days. The model suggests that after repeated dosing at 20 mg ritonavir once daily, the remaining hepatic CYP3A4 activity was approximately 23 % [29]. A maximal reduction to almost 2% was predicted already after the second and third administration of 100 mg ritonavir (and of higher doses). This model thus indicated that it is not possible to reach a maximum inhibition of hepatic CYP3A4 activity with chronic once-daily dosing at 20 mg ritonavir. However, at all the simulated dose levels, it was assessed that the gut CYP3A4 activity was reduced to approximately 4.3–5.7 % after the first administration of ritonavir, even at the lowest dose (20 mg). This indicates that intestinal CYP3A4 is more susceptible to the inhibiting effects of ritonavir compared to the liver after a single low-dose oral administration [29]. This is not surprising, as the gut is first exposed to oral ritonavir and likely at the highest concentrations.

Mathias et al. described that the estimated local intestinal (i.e., luminal) concentration of ritonavir is approximately ~80 μg/mL (average gastrointestinal volume = 250 mL) after a 20 mg dose [25]. Hence, the portal vein concentrations (assuming absorption is complete) over the range of absorption rate constants of 0.3–1.16/h after this dose are similar to, or as much as 3-fold higher than, the previously described K_I (the dissociation constant of the inhibitor for the enzyme) of ~0.123–0.274 μg/mL for almost one hour after administration [25,30,31]. This suggests that the presystemic (between intestine and liver) concentration of ritonavir at a dose of 20 mg (AUC_{tau} = 134 ± 54.9 ng·h/mL) are maintained for at least an hour and the exposure time is sufficient for adequate inactivation of CYP3A [25].

The studies discussed above suggest that lower doses of ritonavir could still be used for the clinical (partial) boosting of other CYP3A-metabolized drugs. There is already a quite high degree (approximately 66% reduction of baseline activity) of inhibition of CYP3A activity observed at an oral dose of 20 mg ritonavir [25]. This is an important finding, because the standard boosting dose of ritonavir used under clinical conditions is much higher (usually 100–200 mg once or twice daily, see Table 1). If complete inhibition of CYP3A is not really necessary, then it may be better to apply a lower dose of ritonavir, thus not exposing individuals unnecessarily to a higher dose, and perhaps limiting or preventing possible side effects of ritonavir and/or of the targeted substrate drug. However, it would be advisable to use therapeutic drug monitoring (TDM) when applying a low boosting dose of ritonavir, in order to adjust to sufficiently high trough levels of the target drug. Depending on the circumstances, TDM may lead one to opt for either a dose elevation of the victim drug or of ritonavir itself.

5. CYP3A recovery after ritonavir administration

Another interesting question during the use of ritonavir as a booster is: What is the recovery time of CYP3A activity after the last administration of ritonavir? In the literature, there is substantial variation reported in this recovery time (washout period), but likewise there was a lot of variation in the ritonavir doses used.

Eichbaum et al. experimentally assessed that substantial CYP3A inhibition (>50%) will hold for more than one day after a single oral 100 mg ritonavir administration [12]. Other clinical studies even found inhibition of CYP3A for three days or longer, which is possibly due to the irreversible mechanism-based inhibition by ritonavir of the enzyme. For example, Katzenmaier et al. demonstrated that the AUC of oral midazolam was still increased three days after ritonavir discontinuation [26]. Even the onset of CYP3A recovery started only two days after the last ritonavir administration, and only 27% of baseline CYP3A activity (starting from 12.5 %) was reached after three days [26]. An important remark with this study is that it used a boosting dose of 300 mg twice daily, which is two or three times the normally used boosting doses of ritonavir (Table 1). As CYP3A will have been fully inhibited, decreasing ritonavir clearance, it may have taken some time from the last

administration (possibly one or two days), before ritonavir levels dropped below levels able to still affect the activity of newly synthesized CYP3A. In fact, measured ritonavir plasma levels 27 h after the last administration (in a 9-day administration regimen) were still 0.19 μg/mL, or 0.264 μM, which is well above the in vitro IC_{50} for CYP3A inhibition (70–130 nM) [26]. Given the irreversible nature of mechanism-based inhibition of CYP3A, it is likely that at this point in time still virtually all CYP3A in the body was inactivated. Furthermore, observations were only continued for three days, so no conclusion could be reached on the full recovery time of CYP3A after terminating high-dose ritonavir usage. In contrast, other studies observed that three (or 3.5) days after discontinuation of oral ritonavir dosed at 200 mg twice daily was sufficient to achieve nearly complete recovery of CYP3A activity using oral triazolam as probe drug [32,33].

Based on the published results, it is difficult to reliably establish the actual recovery time of CYP3A after ritonavir administration. This is especially the case as most published data on this subject utilized higher ritonavir doses than the typical boosting doses (Table 1). The recovery time of CYP3A will certainly be more than one day in most cases, but probably this can be considerably longer based on the aforementioned studies utilizing higher ritonavir doses. The duration of inhibition and recovery of CYP3A will be highly dependent on the turnover rate of enterocytes, which are normally fully replaced within a period of 3.5 days on average [34]. The enterocytes need to have a steady state expression of CYP3A, so the turnover rate of CYP3A itself will be probably somewhat faster than the enterocyte turnover rate. However, this is relatively hard to establish in humans, but possibly further research could clarify this. However that may be, the above described data do indicate that using more modest booster doses of ritonavir, while allowing nearly full CYP3A inhibition, would allow a considerably faster recovery of CYP3A activity, which may offer more flexibility in certain circumstances, and thus perhaps be safer.

6. Influence of ritonavir on hepatic and intestinal CYP3A in humans

Another interesting question is whether oral ritonavir has the same impact on intestinal CYP3A compared to hepatic CYP3A. As stated in an early study of Koudriakova et al. (1998), ritonavir is rapidly metabolized by CYP3A in the liver as well as the small intestine [35]. The formation of one of the main metabolites of ritonavir (*N*-demethylated derivative) is catalyzed by both CYP3A4 and CYP2D6 [35,36]. However, whereas CYP2D6 contributes significantly to the metabolism of ritonavir in the liver, it has no contribution to its biotransformation in the intestine [35].

Furthermore, Kirby et al. showed that ritonavir (400 mg b.i.d. orally administered) significantly decreased hepatic as well as intestinal CYP3A activity in healthy participants [27]. However, their study did not reveal detectable net induction of hepatic and intestinal CYP3A activity after 14 days of treatment with ritonavir (in steady state, see Table 2). Twelve hours after the last ritonavir dose, they observed a remaining reduction of the intestinal CYP3A activity by 78 % (so 22 % remaining activity relative to baseline), which is consistent with normal intestinal enzyme recovery over a 12-h dosing interval [27,34]. They hypothesized that if the synthesis of intestinal CYP3A (for instance by activation of PXR) was induced as little as 3-fold, there would have been recovery to approximately 90 % of baseline CYP3A activity. Therefore, these data suggested that, either there is minimal or no intestinal induction of CYP3A expression by ritonavir in humans, or systemic exposure to ritonavir is still able to inactivate intestinal CYP3A [27]. In view of the irreversible nature of CYP3A inhibition by ritonavir and the high ritonavir doses used in this study (400 mg twice daily), this latter possibility should not be discounted.

Kharasch et al. (2008) investigated the effects of ritonavir on CYP3A and the important drug transporter P-glycoprotein (P-gp) in healthy participants (n = 12, see Table 2) [24]. They studied the short-term (2 days) and steady state (2 weeks) effects of ritonavir (600 mg/day orally on day 1–7; 800 mg/day on day 8–21), using the CYP3A4/5 substrate alfentanil (15 μg/kg IV and 43 μg/kg oral) and the P-gp substrate fexofenadine (60 mg orally) as probe drugs. Their results indicated that there was 72 % inhibition of the hepatic alfentanil clearance and hence CYP3A activity by ritonavir during steady state, which is reached after approximately two weeks of daily ritonavir administration, partly as a consequence of its auto-induction capacity [13,24]. However, they observed a 96 % and 90 % inhibition of the first-pass (oral) alfentanil clearance and hence CYP3A activity by a short-term dose (200 mg t.i.d.) and steady-state higher dose (400 mg b.i.d.), respectively, of ritonavir [24]. Ritonavir in steady state (400 mg b.i.d.) decreased the hepatic extraction ratio of alfentanil from 0.26 to 0.07, the intestinal extraction ratio from 0.51 to zero, and increased the oral bioavailability from 37 % to 95 %. This shows that ritonavir is able to inhibit both hepatic and especially intestinal CYP3A very efficiently. The observation that the intestinal extraction ratio of alfentanil was zero made it, according to the authors, more likely that any upregulation of CYP3A took place primarily in the liver [24]. However, it may also be that inhibition of intestinal CYP3A, even when induced, by ritonavir is extremely efficient due to the high and immediate exposure to oral ritonavir. Fig. 2 shows a graphic (model) prediction of the net hepatic activity of CYP3A, in which the contribution of inhibition, inactivation and induction by ritonavir is included.

The P-gp substrate drug fexofenadine was tested in the same settings. There was a nearly 3-fold increase in fexofenadine $AUC_{0-\infty}$ after the short-term 300 mg twice daily dose of ritonavir, and a 1.4-fold increase in fexofenadine $AUC_{0-\infty}$ with the steady-state 400 mg twice daily dose. This indicated a significantly inhibited P-gp activity in the intestine and/or liver by short-term ritonavir, which was likely partly counteracted by P-gp induction upon long-term ritonavir administration [24]. Thus, ritonavir can also have a major impact on P-gp activity.

7. Impact of potent CYP3A induction on ritonavir efficacy as booster

On the other hand, there are also potent CYP3A inducers, of which the well-known St. John's wort is one of the most effective, depending on the concentration of hyperforin, the main inducing compound in the used preparations. The impact on CYP3A activity of chronic combined administration of St. John's wort (300 mg t.i.d. oral) and ritonavir (300 mg b.i.d. oral) was assessed in a randomized controlled trial by

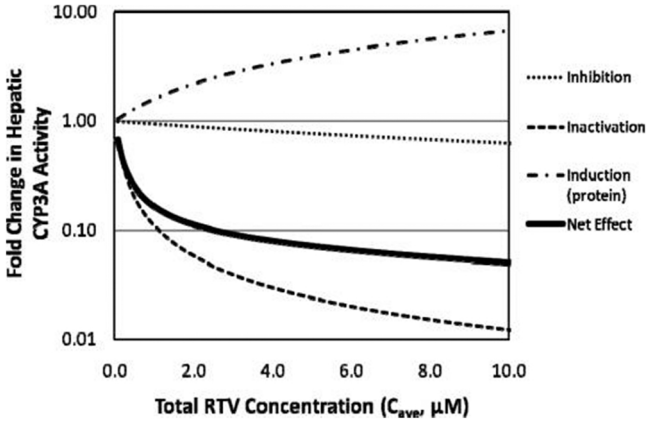

Fig. 2. : The predicted net hepatic CYP3A activity at different ritonavir concentration levels based on data from the clinical study of Kirby et al. (2011). Ritonavir administration will result in net inactivation of hepatic CYP3A activity, which is irrespective of simultaneous or staggered administration with a CYP3A substrate drug. The contribution of ritonavir-dependent inhibition, inactivation and induction of hepatic CYP3A is included in the predicted net activity. RTV, ritonavir; C_{aver}, average concentration.
This Figure was reproduced with permission from [27].

Hafner et al. in twelve healthy non-smoking participants. Midazolam was used as probe drug and participants received first a fixed oral dose of 4 mg midazolam, and subsequently 6 h later they received a dose of 2 mg intravenously. This study showed that the combination of ritonavir and St. John's wort still resulted in predominantly CYP3A inhibition, at least in the short term [37]. Even after 14 days of coadministration, ritonavir profoundly reduced both hepatic and intestinal CYP3A activity, increasing the plasma AUC of oral midazolam from baseline (no ritonavir or St John's wort) by at least 4-fold, in spite of likely very marked induction of CYP3A [32,37]. Indeed, 48 h after termination of the ritonavir and St. John's wort coadministration, the oral midazolam AUC was reduced by 16-fold, and still 3.9-fold lower than the baseline value. This testifies to the pronounced lingering induction of CYP3A, as well as the efficacy of the ritonavir-mediated inhibition of this induced CYP3A during the coadministration phase [37]. It is worth noting that this study shows that within 48 h of terminating quite high-dose ritonavir administration (300 mg twice daily), there was very strong resumption of CYP3A activity, illustrating that irreversible CYP3A inhibition in humans can be at least partly resolved within two days. However, likely the high induction of CYP3A itself also speeds up this process, so it may be difficult to extrapolate these data to CYP3A recovery times from ritonavir in a non- or mildly induced situation [37].

8. Gene induction capacity of ritonavir and its consequences

In addition to ritonavir's strong inactivation capacity of CYP3A, a number of studies showed that ritonavir is also able to inhibit many drug transporters, such as P-gp/ABCB1 (as also mentioned earlier), breast cancer resistance protein (BCRP/ABCG2), multidrug resistance-associated protein 2 (MRP2/ABCC2), and organic anion-transporting polypeptide (OATP/SLCO) transporters, albeit at comparatively high concentrations [38–41]. P-gp, BCRP, and MRP2 are efflux transporters expressed at the apical membrane of enterocytes in the small intestine and in the bile canalicular membrane of hepatocytes, where they can restrict the oral availability of their substrates. OATPs are primarily uptake transporters expressed at the basolateral membrane of hepatocytes [41].

Moreover, as is the case for several other HIV protease inhibitors, ritonavir is also able to somewhat induce (increase) its own clearance, possibly in part due to net induction of CYP3A expression, during the course of a chronic administration regimen [27,28]. However, after a few doses of ritonavir the CYP3A activity itself is already reduced to approximately 10% of baseline [27,28]. This suggests that there must have been induction of other ritonavir clearance mechanisms, probably other P450 enzymes and/or efflux pumps, such as P-gp or MRP2 [28].

In addition, a study by Culm-Merdek et al. (2006) in healthy participants (n = 23) showed that administration of ritonavir (200 mg b.i.d. oral) caused a modest (2-fold) increase in the AUC of oral triazolam (0.1875 mg orally), including a prolongation in the half-life and a decrease in oral clearance [32]. Triazolam is a substrate for CYP3A, but not for efflux transporters, which makes it unlikely that impairment of enteric efflux transport was involved in these shifts [32]. As described earlier, ritonavir is also able to induce CYP3A somewhat in the chronic phase, which is on the gene/RNA level and less prominent on the activity level of CYP3A protein. The latter is probably masked by the inhibitory effects of ritonavir.

Furthermore, Kirby et al. showed that ritonavir is able to significantly induce CYP1A2, CYP2B6, CYP2C9, and CYP3A4 expression in healthy participants at a 400 mg twice daily dosing [28]. They predicted a $\leq$ 30 % induction of CYP1A2, CYP2B6, or CYP2C9 at a dose of 100 mg twice daily, which would be relevant when ritonavir is used as a booster [28]. As discussed above, ritonavir possesses the ability to activate the pregnane X receptor (PXR), which induces the transcription of CYP3A and various other xenobiotic detoxification genes [27,42]. PXR and CAR are nuclear receptors activated by binding of many xenobiotics that regulate transcription of a range of detoxification genes, including P-gp. Ritonavir is not a ligand for at least one tested CAR splice variant (CAR3), which makes it more likely that induction of CYP-enzymes, such as CYP2B6, is PXR-mediated [28,43]. Additionally, induction of CYP2C19 and uridine diphosphate-glucuronosyltransferase (UGT) by ritonavir has been described [18,44,45]. Ritonavir thus exhibits a number of gene induction capacities. However, as mentioned before, with respect to CYP3A activity, the inhibition or inactivation capacity clearly predominates [46].

9. Comparison of ritonavir with cobicistat, another strong CYP3A inhibitor

It is of course important to know whether ritonavir is the best choice for the use of a CYP3A-inactivating booster in various clinical circumstances, from the perspectives of safety, efficacy, reproducibility and reliability. In the clinical setting, there are also other drugs, such as the ritonavir analog cobicistat (Fig. 1), used as pharmacokinetic booster to enhance oral availability of CYP3A substrate drugs. Previously in drug-drug interaction studies, ketoconazole was routinely used as an index inhibitor, as it is a relatively specific, high-affinity, and reversible CYP3A inhibitor. Nowadays, however, there is a limited use for ketoconazole, because of the purported risk of rare but severe liver injury caused by this compound, potentially necessitating liver transplantation [47]. Although this liver injury seems to be very uncommon and mostly asymptomatic and reversible, both FDA and EMA advise against the use of ketoconazole as a CYP3A inhibitor [48].

More recently, cobicistat has been approved (in 2014) as a dedicated CYP3A inhibitor drug for pharmacokinetic boosting [47,49]. The chemical structure of cobicistat is very similar to that of ritonavir (Fig. 1), and it exhibits a very similar inhibitory potency of CYP3A [49,50]. Hossain et al. showed in a validated human liver microsomal (HLM) model that both ritonavir and cobicistat are potent inhibitors of CYP3A [49]. In fact, ritonavir (IC_{50} = 0.015 μM) was a slightly better inhibitor compared to cobicistat (IC_{50} = 0.032 μM). Both drugs showed a time-dependent inhibition, so longer incubation with the HLMs leads to a higher inhibitory potency by a factor of 2- to 3-fold, indicative of irreversible mechanism-based inhibition. In contrast to the claim of the manufacturer that cobicistat is more selective than ritonavir, Hossain et al. found that cobicistat also inhibits CYP2B6, CYP2C19 and CYP2D6 to a similar or even greater extent compared to ritonavir [49].

This was further investigated by Marzolini et al., who presented a useful comparative overview of ritonavir and cobicistat. They observed similar inhibition of CYP3A activity in humans by ritonavir (100 mg once daily) and cobicistat (150 mg once daily), the usual boosting doses [51]. However, cobicistat was not able to inhibit CYP2D6 to the same extent as ritonavir, behaving like a weak inhibitor of this enzyme [18]. Additionally, cobicistat was able to inhibit the intestinal transporters P-gp and BCRP to a similar extent as ritonavir, which can increase the absorption of coadministered P-gp and BCRP substrates [51,52].

Furthermore, both booster drugs are able to inhibit the hepatic OATP uptake transporters and the multidrug and toxin extrusion protein 1 (MATE1), a transporter involved in the tubular secretion of creatinine and many cationic drugs [51,53]. Inhibiting the latter results in a slight increase in serum creatinine levels and a related decrease in estimated glomerular filtration rate. Importantly, however, this effect is mainly related to the direct inhibition of creatinine secretion by MATE1 instead of an actual impairment of the overall renal function, an obvious worry for a boosting drug. Interestingly, the serum creatinine level was higher in a cobicistat regimen compared to a ritonavir regimen [51]. A possible explanation for this is that cobicistat is actively transported into the tubular cells by organic cation transporter 2 and could accumulate, resulting in a higher local concentration of cobicistat able to inhibit MATE1 [51,53].

An important difference between ritonavir and cobicistat concerns their ability to activate PXR, because cobicistat has only limited effects on PXR. This makes it unlikely that cobicistat can substantially induce

other drug-metabolizing enzymes through PXR/SXR (NR1I2) [51,54]. As mentioned earlier, ritonavir activates PXR and through this route it induces CYP1A2, − 2B6, − 2C9 and − 2C19, CYP3A itself, and glucuronidation (UGT) enzymes [51]. Since cobicistat lacks the ability to activate PXR and thus only possesses inhibitory effects, this could be an advantage. Therefore, ritonavir has probably somewhat more drug-drug interaction risks due to its higher ability to induce different enzymes and transporters compared to cobicistat. However, whether this has a significant impact in daily clinical use remains to be seen.

10. Conclusions

Ritonavir is a widely used booster drug due to its CYP3A inactivation capacity. Its mechanism-based inactivation of the enzyme takes time, resulting in increased inhibition after multiple administrations. Interestingly, ritonavir shows non-linear pharmacokinetics, which is probably related to auto-inhibition, i.e., inhibition of its own metabolism by CYP3A, even after a single administration. The CYP3A inactivation is more pronounced in the intestine compared to the liver, likely because intestinal CYP3A is first exposed and to a relatively higher concentration of ritonavir. Furthermore, ritonavir is able to induce CYP3A expression and thereby induce its own clearance, so-called auto-induction. Initially, primarily inhibition of CYP3A takes place, but after multiple administrations, induction of CYP3A will also play a role. This will be on the gene level (mediated by PXR) as well as on the potential activity level of the enzyme. However, as shown by several studies in this review, the inhibition of CYP3A is generally predominant, even after chronic administration of ritonavir.

With lower ritonavir doses, for example 20 mg instead of 100 mg, more CYP3A will remain active and could still contribute to the elimination of ritonavir. Therefore, if there is no need for complete inhibition of CYP3A with respect to exposure to the victim drug, lower doses of ritonavir could be considered, decreasing the risk of adverse events due to ritonavir and/or the concomitant victim drug. For example, when a common side effect of a boosted drug is diarrhea, this risk will increase because this is also one of the most common adverse events of ritonavir itself [55]. Circumstances where it is not necessary (or even desirable) to reach full inhibition could be for instance with (cyto-)toxic victim drugs. Furthermore, the ability (or risk) to inhibit or induce other enzymes and transporters will likely be reduced with lower ritonavir doses as well. On the other hand, a possible risk is that potent CYP3A inducers could possibly overrule the inhibitory effects of lower ritonavir doses, resulting in an overall increase in CYP3A activity. For these reasons, with lower ritonavir boosting doses, therapeutic drug monitoring (TDM) might be advisable to obtain sufficiently high trough levels of the CYP3A victim drug.

Surprisingly, there is no consensus about the exact recovery time of CYP3A after discontinuation of ritonavir, although it is clear that its inhibitory effects will at least hold for 1 day. Nearly full recovery of CYP3A is observed after approximately three days after discontinuation of a typically used boosting dose, which is consistent with the enterocyte turnover rate. When lower booster doses are applied, this recovery time will probably be shorter.

Some concern about the drug-drug interaction profile of ritonavir has led to the development of cobicistat. In contrast to the claim of its manufacturers, it seems unlikely that cobicistat is a significantly more potent CYP3A inhibitor than ritonavir. Although cobicistat appears to mostly lack the capacity to induce other CYP enzymes (including CYP3A), because it lacks the capacity to activate PXR, it could still inhibit other enzymes and drug transporters in a similar way as ritonavir. Therefore, unwanted drug-drug interactions, although reduced, can still occur with this pharmacokinetic enhancer. All in all, ritonavir is, despite its fairly complicated in vivo pharmacology, still one of the most potent CYP3A inhibitors known, and its wide use as a booster to enhance the bioavailability of CYP3A substrate drugs still seems fully warranted, given its apparent safety.

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CRediT authorship contribution statement

Nancy H.C. Loos: Conceptualization; Visualization; Writing – original draft. Jos H. Beijnen: Supervision. Writing – review content. Alfred H. Schinkel: Conceptualization; Supervision. Writing – review & editing. All authors commented on and approved the manuscript.

Declaration of Competing Interest

Jos H. Beijnen is part-time employee and (indirect) stockholder of Modra Pharmaceuticals, a small spin out company that develops oral taxane formulations boosted with ritonavir. Jos H. Beijnen is also (co-) inventor on a patent of oral taxane formulations. The other authors of this manuscript have no competing interests to declare.

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