Interaction of Rifampicin and Darunavir/Ritonavir or Darunavir/Cobicistat *In Vitro*

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Running Title: Interaction of Rifampicin with Boosted Darunavir

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ABSTRACT

Treatment of HIV patients co-infected with tuberculosis (TB) is challenging due to drug-drug interactions (DDIs) between antiretrovirals (ARVs) and anti-TB drugs. The aim of this study was to quantify the effects of cobicistat (COBI), or ritonavir (RTV), in modulating DDIs between darunavir (DRV) and rifampicin (RIF) in a human hepatocyte-based *in vitro* model. Human primary hepatocyte cultures were incubated with RIF alone, or in combination with either COBI or RTV for three days, followed by co-incubation with DRV for one hour. Resultant DRV concentrations were quantified by HPLC-UV, and the apparent intrinsic clearance ($CL_{int.app.}$) of DRV was calculated. Both RTV and COBI lowered RIF-induced increases in $CL_{int.app.}$ in a concentration-dependent manner. Linear regression analysis showed that $\log_{10}$ RTV and $\log_{10}$ COBI concentrations were associated with percentage inhibition of RIF-induced elevations in DRV $CL_{int.app.}$ β = -94 (95% CI = -108 to -80; P=0.0001), and β = - 61 (95% CI = -73 to -49; P=0.0001), respectively. RTV was more effective in lowering 10 µM RIF-induced elevations in DRV $CL_{int.app.}$ (EC_{50} = 1.54 µM) than COBI (EC_{50} = 2.58 µM). Incubation of either RTV, or COBI, in combination with RIF was sufficient to overcome RIF-induced elevations in DRV $CL_{int.app.}$, with RTV more potent than COBI. These data provide the first *in vitro* experimental insight into DDIs between RIF and COBI-boosted or RTV-boosted DRV, and will be useful to inform physiologically-based pharmacokinetic models to aid in optimising dosing regimens for the treatment of HIV-TB patients receiving concomitant ARVs and anti-TB drugs.

INTRODUCTION

Approximately 25% of human immunodeficiency virus-1 (HIV)-infected patients worldwide are co-infected with *Mycobacterium tuberculosis* (1, 2), accounting for 390,000 deaths in 2014 (3). Clinical management of HIV-tuberculosis (HIV-TB) patients presents significant challenges, especially in resource-limited settings (2, 4), where virological failure or intolerance to first-line antiretroviral therapy requires the use of HIV protease inhibitors (PIs) (5). PIs largely undergo phase I metabolism by cytochrome p450 3A4 (CYP3A4), and are also substrates of P-glycoprotein (P-gp; ABCB1) (6). Consequently, PIs are commonly administered in combination with pharmacokinetic (PK) "boosters" such as ritonavir (RTV) or cobicistat (COBI), which act by inhibiting CYP3A4-mediated PI metabolism and P-gp-mediated PI efflux, thereby improving the PK profile of PIs by prolonging PI half-life, and increasing PI bioavailability (7-9).

Rifampicin (RIF) is an essential component of short-course anti-TB treatment regimens (2, 10); however, RIF is also a potent inducer of the expression and activity of several metabolic enzymes – including CYP3A4 (11). Co-administering RIF with PIs can result in clinically-significant drug-drug interactions (DDIs), whereby PI bioavailability may be significantly reduced (>75%) (10, 12-14). Consequently, administering standard-doses of RTV-boosted PIs to HIV-TB patients receiving RIF is contraindicated under the current World Health Organisation (WHO) guidelines (15). The search for effective second-line therapeutic options for the treatment of HIV-TB co-infected patients is therefore a research priority (16).

Darunavir (DRV) is chiefly metabolised by CYP3A4 (17) and co-administration of a low-dose of either RTV or COBI together with DRV increases DRV systemic bioavailability

(18, 19). In addition, the high barrier to genetic resistance, as well as the tolerability, safety profile, and potency of DRV - when administered in combination with a low-dose of either RTV (DRV/r), or COBI (DRV/c) - have made these fixed-dose combinations important options for the treatment of HIV-patients (20-22).

Previous studies have demonstrated markedly reduced exposure of RTV-boosted PIs, including atazanavir (ATV) (12), indinavir (IDV) (13), and lopinavir (LPV) (14), as well as an increased risk of hepatotoxicity when RIF is co-administered with these drugs in healthy volunteers. For this reason, studies aimed at investigating DDIs between DRV/r and RIF in HIV-negative subjects have not been undertaken. Similarly, the extent of the DDI between DRV/c and RIF remains unknown. A recent population PK (pop-PK) analysis showed that it was possible to offset the effects of RIF on DRV C_{trough} by increasing the dose of DRV/r administered (23); raising the possibility that RTV may overcome potential DDIs between DRV and RIF *in vitro* and *in vivo*. The aim of the present study was to quantify - using an *in vitro* model - the extent of DDIs arising from co-incubation of RIF with either RTV or COBI, by specifically measuring the apparent intrinsic clearance ($CL_{int.app.}$) of DRV by primary human hepatocytes.

MATERIALS AND METHODS

Chemicals. DRV (Cat. No.: S1620) and COBI (Cat. No.: S2900) were purchased from Selleckchem (Munich, Germany). RIF (Cat. No.: R3501), RTV (Cat. No.: SML0491), potassium phosphate monobasic (Cat. No.: P0662), Hanks' balanced salt solution (Cat. No.: H8264), methanol (Cat. No.: 34860), and acetonitrile (Cat. No.: 34967) were purchased from Sigma-Aldrich (Poole, UK). Orthophosphoric acid (Cat. No.: 153154D) was purchased from VWR (Lutterworth, UK). HPLC-grade water was produced by an ELGA PureLab system (Veolia Water Technologies, High Wycombe, UK).

Primary Hepatocytes. Cryopreserved primary human hepatocytes were purchased from Life Technologies (Cat. No.: HMCPIS; Inchinnan, Scotland). Hepatocytes from a total of four donors were used (**Table 1**).

Stock Solutions. Stock solutions of COBI, DRV, RIF and RTV were freshly prepared in 100% (v/v) methanol at concentrations 6443, 1684.3, 15000 and 6935.4 µM respectively. Prior to use in experiments, all stock solutions were sterile-filtered through a Millex 0.22 µm polyethersulfone membrane (Millipore, Cat. No.: SLGP033RS; Watford, UK), and were either used immediately, or were stored at -20 °C for up to five days prior to use.

Concentrations of drugs used in this study. Primary cryopreserved human hepatocytes were treated with a range of concentrations of test compounds - COBI (0.13—12.76 µM), RIF (0.50—20.00 µM) and RTV (0.01—10.00 µM) - spanning the therapeutic plasma concentration range in humans as determined from clinical PK data (24), (25). The concentration of DRV used in

98 experiments (5 µM), was selected from a value within the therapeutic range of DRV, as obtained
99 from clinical PK data (18).

100 **Culture of Primary Human Hepatocytes.** Primary cryopreserved human hepatocytes were
101 thawed in Cryopreserved Hepatocyte Recovery Medium (CHRM®, Life Technologies, Cat. No.:
102 CM7000) and were re-suspended in Williams' Medium E (WME) plating medium (WME Life
103 Technologies, Cat. No.: A1217601, supplemented with Hepatocyte Plating Supplement Pack,
104 Life Technologies, Cat. No.: CM3000). Cell viability was determined using a NucleoCounter®
105 NC-100™ (Sartorius Ltd., Epsom, UK). Viable cells were plated on collagen-coated 96-well cell
106 culture plates (Life Technologies, Cat. No.: CM1096) at a density of 6.5 x 10^4 cells per well in
107 110 µl of WME plating medium. Hepatocytes were incubated in a humidified incubator at 37 °C
108 containing 5% (v/v) CO_2 for five hours prior to removal of the WME plating medium, and
109 overlaying the hepatocyte monolayer with 70 µl per well of Geltrex™ LDEV-Free Reduced
110 Growth Factor Basement Membrane Matrix (Life Technologies, Cat. No.: A1413202) diluted in
111 WME incubation medium (WME Life Technologies, Cat. No.: A1217601, supplemented with
112 Hepatocyte Maintenance Supplement Pack, Life Technologies, Cat. No.: CM4000) to a final
113 concentration of 0.35 mg/ml. Cells were then incubated in a humidified incubator at 37 °C
114 containing 5% (v/v) CO_2 for 24 hours, prior to removal of the WME incubation medium and
115 replacement with 110 µl of fresh WME incubation medium containing test compounds: COBI
116 (0.128—12.76 µM), RTV (0.01—10 µM), RIF (0.5—20 µM) or methanol (0.3% v/v; vehicle
117 control). At 24 hours, and 48 hours post-initial treatment, WME incubation medium was
118 removed, and replaced with fresh WME incubation medium containing test compounds. At 72

hours post-initial treatment cells were treated with test compounds together with DRV (5 µM) for 60 minutes.

Quantification of Darunavir by HPLC-UV. Following 60 minutes of incubation of hepatocytes with test compounds together with 5 µM DRV, 100 µl of WME incubation medium was removed from each well and was transferred to Corning® Pyrex® 75 x 12 mm borosilicate glass tubes (Appleton-Woods, Cat. No.: KC350) containing 300 µl of 100% acetonitrile. Standards and quality control samples were prepared in WME incubation medium and were treated in the same way. All samples were then vortexed for five seconds, and were dried in a Jouan RC10.22 vacuum centrifuge for six hours at room temperature (18—25°C). After drying, samples were re-constituted in 330 µl of 20% (v/v) acetonitrile and 80% (v/v) H_2O. One hundred microlitres of the resultant suspension was used to quantify DRV by HPLC-UV.

Chromatographic separation of DRV was achieved using a Waters Atlantis T3 (4.6 x 100 mm, 3 µm) column (Waters, Elstree, UK) equipped with a 10 x 4 mm, 3 µm Fortis C18 Guard (Fortis™ Technologies Ltd., Chester, UK). A Dionex P680 HPLC pump, Dionex ASI-100 automated sample injector and a Dionex UVD170U UV detector (Thermo Fisher Ltd., Hemel-Hempstead, UK) were used. Mobile phases C (25 mM KH_2PO_4, pH 3.3/orthophosphoric acid) and D (100% acetonitrile) were used in a step-gradient elution as follows: 70% C/30% D from 0.0 to 1.5 min, 35% C/65% D from 1.5 to 7.0 min, 20% C/80% D from 7.0 to 9.5 min and 70% C/30% D from 9.5 to 12.5 min. Elution was carried out at room temperature (18—25°C), and the flow rate was maintained at 1.00 ml/min. Chromatograms were analysed and DRV was quantified at 267 nm using Chromeleon software (version 6.8; Thermo Fisher Ltd.). Each experimental condition was assessed in triplicate. The lower limit of detection (LOQ) of DRV

was determined to be 0.156 µM. The assay was linear between 0.156 µM and 10 µM (upper LOQ). The mean coefficient of variability (CV) of intra-day precision was 2.6%, whilst the mean CV of intra-day accuracy was 2.0%. The mean CV of inter-day precision was 2.2%, and the mean CV of inter-day accuracy was 1.2%. The mean recovery of DRV from WEM was 96.1%.

Calculation of $CL_{int.app.}$ of Darunavir in Hepatocytes. Apparent intrinsic clearance ($CL_{int.app.}$) of DRV was calculated based on a method described previously (26). This is summarised in **Equation 1**:

Equation 1: $CL_{int.app.} = (\ln 2 / \textit{in vitro}\ t_{1/2}) \times (\mu l \text{ incubation volume} / 10^6 \text{ hepatocytes})$

Results were expressed as the mean ± SD (µl/min/10^6 hepatocytes) of a total of three donors per condition tested. Three biological replicates were quantified per condition tested, using hepatocytes obtained from three separate donors in each case.

Data and Statistical Analysis. Statistical analyses were carried out using IBM® SPSS® Statistics (Version 22; IBM Corporation, Armonk, NY, USA). All data were assessed for normality using a Shapiro–Wilk test. Univariate and stepwise-elimination multivariate linear regression analyses were conducted to characterise the influence of co-incubating primary human hepatocytes with various concentrations of RTV or COBI together with RIF on DRV $CL_{int.app}$ Effective concentration (EC_{50}) was calculated using GraphPad Prism® (Version 5; GraphPad Software, Inc. La Jolla, CA, USA).

RESULTS

Assessment of the $CL_{int.app.}$ of Darunavir Following Combination Incubation of Primary Human Cryopreserved Hepatocytes with Ritonavir and Rifampicin. Primary human hepatocytes are commonly used as a tool to predict hepatic metabolic clearance of xenobiotics and DDIs *in vitro* (27, 28). Using this model system, the $CL_{int.app.}$ of DRV was initially calculated under control conditions in which hepatocytes (Lot HU1399, Lot HU1587 and Lot HU1621) were incubated with DRV alone. Under these conditions, mean DRV $CL_{int.app.}$ was 10.5 ± 3.8 µl/min/10^6 hepatocytes (*n*=3). Incubation of hepatocytes with RIF was sufficient to markedly increase DRV $CL_{int.app.}$ at each concentration of RIF tested (0.5—20 µM) (**Fig. 1**). The maximal RIF-induced increase (1.9 ± 0.3-fold; *n*=3) in DRV $CL_{int.app.}$ was observed with 10 µM RIF (**Fig. 1**).

Co-incubation of RIF with RTV reduced 10 µM RIF-induced increases in $CL_{int.app.}$ in a RTV concentration-dependent manner (**Fig. 1**). Notably, RTV (1 µM) was sufficient to overcome the effect of 10 µM RIF on DRV $CL_{int.app.}$, reducing DRV $CL_{int.app.}$ to 0.78 ± 0.25-fold – equivalent to -22% when compared to control levels in which cells were treated with DRV alone (*n*=3; **Fig. 1**). Increasing RIF concentrations above 10 µM (12.5—20 µM) did not impact the effectiveness of RTV to overcome RIF-elevated DRV $CL_{int.app.}$ (**Fig. 1**). Specifically, 1 µM RTV lowered 12.5 µM RIF-induced and 20 µM RIF-induced DRV $CL_{int.app.}$ by 55% and 47%, to (8.6 ± 3.2 µl/min/10^6 hepatocytes; *n*=3) and (8.8 ± 3.4 µl/min/10^6 hepatocytes; *n*=3), respectively.

Assessment of the $CL_{int.app.}$ of Darunavir Following Combination Incubation of Primary Human Cryopreserved Hepatocytes with Cobicistat and Rifampicin. In a separate set of experiments, human hepatocytes from three individual donors (Lot HU1399, Lot HU1574 and Lot HU1587) were used to determine the effects of incubating rifampicin together with cobicistat on DRV $CL_{int.app.}$. Under control conditions, where primary human cryopreserved hepatocytes were incubated with DRV alone, DRV $CL_{int.app.}$ was 13.2 ± 1.8 µl/min/10^6 hepatocytes, (n=3). Incubation of hepatocytes with RIF (0.5—20 µM), induced a mean increase in DRV $CL_{int.app.}$ of 55.8%. In cells treated with 1 µM RIF, co-incubation with the lowest concentration of COBI tested (0.42 µM) was effective in lowering RIF-induced DRV $CL_{int.app.}$ by 36.9%, yielding a DRV $CL_{int.app.}$ of 12.2 ± 2.8 µl/min/10^6 hepatocytes (n=3). Hepatocytes treated with 10 µM RIF exhibited a DRV $CL_{int.app.}$ of 21.6 ± 2.6 µl/min/10^6 hepatocytes (n=3). COBI induced a concentration-dependent attenuation of the DRV $CL_{int.app.}$, elicited by 10 µM RIF, with 1.28 µM COBI being sufficient to lower DRV $CL_{int.app}$ to 11.6 ± 2.6 µl/min/10^6 hepatocytes (n=3), 13% below DRV control levels (**Fig. 2**). COBI was also effective at reducing $CL_{int.app.}$ elevations induced by higher concentrations of RIF, as co-incubation with 1.28 µM COBI reduced 20 µM RIF-elevated DRV $CL_{int.app.}$ by 46% (12.4 ± 3.9 µl/min/10^6 hepatocytes; n=3).

Comparison of Cobicistat- and Ritonavir-mediated Reduction of Rifampicin-Induced Darunavir $CL_{int.app.}$. To compare the relative effectiveness of RTV and COBI to attenuate RIF-induced increases in DRV $CL_{int.app.}$, the percentage inhibition of 10 µM RIF-induced elevations in DRV $CL_{int.app.}$ achieved by co-incubation with either COBI (0.13—12.76 µM), or RTV (0.1—10 µM), was determined in comparison to control conditions where cells were treated with 10 µM RIF alone (**Fig. 3**). The effective concentration 50% of maximum response (EC_{50}) of COBI and RTV calculated from the percentage-change in DRV $CL_{int.app.}$ under these conditions was 1.5 µM for COBI and 2.6 µM for RTV (**Fig. 3**). In addition, the maximal inhibition of 10 µM RIF-induced elevations achieved by COBI and RTV were different, with RTV resulting in a 69.5% inhibition of 10 µM RIF-induced increases in DRV $CL_{int.app.}$, whilst COBI-mediated reduction in 10 µM RIF-induced increases in DRV $CL_{int.app.}$ was 56.9% (P=0.05).

Following data normalisation, linear regression analysis of the effects of RTV and COBI in combination with RIF at each concentration tested on the percentage change in DRV $CL_{int.app.}$ showed an association between $\log_{10}$ RTV concentrations, and $\log_{10}$ COBI concentrations and percentage inhibition of RIF-induced DRV $CL_{int.app.}$ of β = -94 (95% CI = -108 to -80; P=0.0001), and β = - 61 (95% CI = -73 to -49; P=0.0001), respectively. Conducting linear regression analysis of the effects of RIF on DRV $CL_{int.app.}$ revealed that RIF exerted a similar effect on DRV $CL_{int.app.}$ in the two independent sets of RTV and COBI experiments, with a positive association observed between RIF concentration and DRV $CL_{int.app.}$ of β = 22 (95% CI = 9 to 35; P=0.001) and β = 16 (95% CI = 5 to 27; P=0.004) in the RTV experiments, and COBI experiments, respectively.

DISCUSSION AND CONCLUSIONS

RIF strongly induces the expression of metabolic enzymes such as CYP3A4 (29-31), and can also induce the activity of drug transporters (32). Collectively, this can result in clinically-relevant DDIs in patients that receive RIF together with other medications (11, 33). These DDIs present challenges for the treatment of HIV-TB patients, as several therapeutic options are contraindicated due to known DDIs (10), whilst other potentially viable treatment regimens may either be delayed, or avoided completely due to hypothetical DDIs that are predicted to occur between anti-TB drugs and ARVs such as PIs. For example, co-administering the standard-dose of any PI with RIF is currently contraindicated under WHO guidelines (15), but the extent of potential DDIs between RIF and PIs has not been determined for all PIs, including DRV. Co-administering dose-adjusted LPV/r, or SQV/r together with RIF is indicated, albeit with the caveat that high levels of toxicity can occur. This raises the possibility that administering other PIs, such as RTV-, or COBI-boosted DRV, together with RIF may also be feasible. The present study addresses this issue by providing the first experimental insight into the effects of co-incubating either RTV, or COBI, together with RIF on DRV $CL_{int.app.}$ in a human hepatocyte-based *in vitro* model of drug metabolism.

Utilisation of human hepatocytes to predict hepatic metabolic clearance of xenobiotics is well-established (27, 28). In this study, incubation of cryopreserved human hepatocytes with RIF increased DRV $CL_{int.app.}$ (**Fig. 1** and **Fig. 2**). This is likely due to induction of CYP3A4 (17, 34), although the effects of RIF on transporters may also be important (28). Uptake transporters such as organic anion transporting polypeptide isoform 1B1 (OATP1B1) (35), and efflux transporters such as P-gp (36), have been shown to play a role in PI elimination, and therefore

may also be relevant in the DDIs between RIF and COBI-, or RTV-boosted DRV. Indeed, RIF has been shown to inhibit OATP1B1 (37), and DRV uptake by OATP1B1 and OATP1B3 in transfected CHO cells has been reported (38). Utilising a pop-PK-model, it has been suggested that OATP3A1 polymorphisms are associated with DRV PK (39), in addition, a recent physiologically-based PK (PBPK) modelling-based study that investigated the PK of DRV/r during pregnancy has also suggested a role for hepatic transporters in DRV disposition (40).

Co-incubation of human cryopreserved hepatocytes with COBI and RIF, or RTV and RIF - using concentrations spanning the *in vivo* therapeutic range of these compounds - revealed that both RTV and COBI could reduce RIF-enhanced DRV $CL_{int.app.}$ in a concentration-dependent manner (**Fig. 1** and **Fig. 2**). RTV was more effective than COBI at attenuating the RIF-induced increase in DRV $CL_{int.app.}$, with RTV exhibiting a lower EC_{50} compared to COBI, whilst RTV also achieved greater maximal inhibition of the 10 µM RIF-induced increase in DRV $CL_{int.app.}$ compared to COBI (**Fig. 3**). Furthermore, regression analysis revealed a stronger effect of RTV in comparison to COBI for their relative contribution in reducing RIF-induced increases in DRV $CL_{int.app.}$. Due to the more recent approval of COBI, data regarding potential DDIs between COBI and other medications is more limited than with RTV. The expected differential DDI profiles of COBI and RTV when administered with co-medications have been recently reviewed (41, 42). RTV and COBI both serve as strong inhibitors of CYP3A4 *in vivo* (43, 44); however, RTV is also known to induce the expression of various metabolic enzymes, including *CYP3A4*, in primary human hepatocytes *in vitro* (30). Very few studies aimed at investigating the relative effects of COBI as an inducer of metabolic enzyme expression have thus far been conducted, although it has been suggested that the induction potential of COBI is less than that of RTV (45),

and that COBI is not expected to induce *CYP3A4* expression (46). It was recently suggested that hepatic uptake of RTV occurs chiefly by passive diffusion (47). In addition, RTV has been shown to induce expression of the efflux transporters *P-gp* (30), and multidrug resistance-associated protein 1 (*MRP1*; *ABCC1*) in primary human hepatocytes *in vitro* (30). DRV is a substrate of P-gp (48) and OATP1A2 and OATP1B1 (35), whilst RTV appears to inhibit P-gp (48), as well as OATP1B1 and OATP1B3 (38), *in vitro.* At the same time, RIF has been described as an inhibitor of various OATPs *in vitro,* including OATP1B1 and OATP1B3 (38). In addition, chronic exposure to RIF has been shown to exert an inhibitory effect on P-gp *in vitro* (49). It remains to be seen therefore what the net contribution of transporters such as OATP1B1, OATB1B3 and P-gp may be on plasma levels of DRV *in vivo,* especially when DRV is administered in combination with other compounds such as RIF.

The PK profiles of DRV/r (800/100 mg, *qd*) and DRV/c (800/150 mg, *qd*) in HIV-infected patients are broadly similar (50, 51). However, in a study conducted in healthy volunteers, it has been reported that DRV C_{min} values were 30% lower in individuals treated with DRV/c compared with individuals treated with DRV/r (52). In addition, PK analysis of the PI tipranavir (TPV), when administered in combination with COBI or RTV in healthy volunteers, showed that TPV AUC, C_{max} and C_{tau} levels were significantly lower with COBI compared to RTV (53). Collectively, these studies suggest that the pharmacoenhancment with COBI is not always equal to that of RTV.

Whilst no studies have been conducted investigating the effects of co-administering either DRV/r or DRV/c with RIF on DRV bioavailability, it has recently been shown using a pop-PK modelling approach that administering dose-adjusted DRV/r (1600/200 mg *qd*; 800/100

mg *bid*; or 1200/150 mg *bid*) can potentially overcome the effects of RIF on DRV C_{trough}, albeit with the caveat that RTV-related side-effects may occur and that a higher pill burden would be required (23). These *in silico* findings are in general agreement with the *in vitro* outcomes of the present study. In addition, it is interesting to speculate that given the observation that low concentrations of either RTV or COBI could overcome RIF-induced elevations in DRV $CL_{int.app.}$, increasing the dose of the pharmacoenhancer may not be necessary to achieve therapeutic concentrations of DRV in combination with RTV or COBI. Even so, extrapolating the *in vivo* significance of *in vitro* data presents multiple challenges (54, 55), and it is difficult to directly infer how these results may translate *in vivo.* For example, increasing the dose of RTV in combination with a given PI is not always sufficient to overcome the effects of RIF. Indeed, a study of the effects of RIF on the steady-state PK of ATV with RTV in healthy volunteers showed that administering ATV/RTV 300/100 mg, ATV/RTV 300/200 mg, and ATV/RTV 400/200 mg was insufficient to completely overcome the inductive potential of RIF 600 mg (12). In an effort to better understand the absorption, distribution, metabolism and elimination of various compounds, the use of PBPK models has recently gained popularity (56). Various PBPK models have been developed that have proven useful in predicting the effects of administering ARVs in HIV patients with co-morbidities (57). Indeed, a recent study described the development of a PBPK model for predicting clinical DDIs from RIF-based *in vitro* human hepatocyte data (58), and it is therefore hoped that the data presented herein will be of use in the development of PBPK models to predict the effects of co-administering boosted PIs with anti-TB drugs.

In conclusion, the results presented herein provide insight into the relative effects of RTV and COBI as pharmacoenhancers of DRV in the presence of RIF in an *in vitro* model of drug metabolism, which can be used in conjunction with PBPK models to rationalise future strategies aimed at optimising treatment regimens. Further work should aim to elucidate the mechanisms that give rise to the differential inhibitory potential of COBI and RTV demonstrated herein, as well as to validate these results *in vivo*. Future studies should also aim to further evaluate the effects of COBI and RTV on gene expression, as well as the effects of these compounds on the expression and activity of various drug transporters *in vitro*. Finally, it would also be of interest to use this model system to evaluate potential DDIs that may occur between RIF and RTV, or COBI, in combination with other PIs, or with other co-medications.

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FIGURES AND FIGURE LEGENDS

FIGURE 1

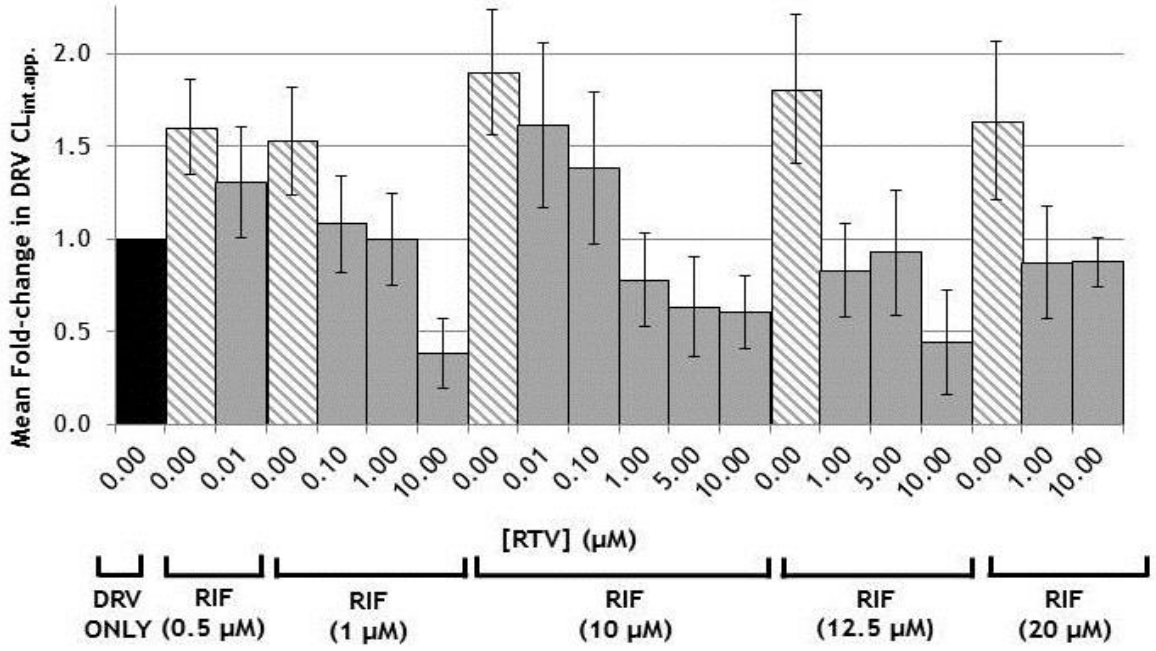

Figure 1: Effects of rifampicin alone, or in combination with ritonavir, on mean DRV $CL_{int.app.}$ in primary human hepatocytes *in vitro*. Cryopreserved primary human hepatocytes were incubated with rifampicin (RIF; 0.5—20 μM), hatched bars; or with ritonavir (RTV; 0.01–10 μM) and RIF (0.5—20 μM), grey bars; each day for 72 hours. All cells were then incubated with RIF (0.5—20 μM), or RIF (0.5—20 μM) together with RTV (0.01–10 μM) as described above, together with darunavir (DRV; 5 μM), black bar, for 60 minutes. Control cells were treated with DRV (5 μM) alone for 60 minutes. The results shown represent the mean DRV $CL_{int.app.}$ from three biological replicates measured in hepatocytes from three independent donors (Lot HU1399, HU1587 and HU1621). Error bars: SD.

FIGURE 2

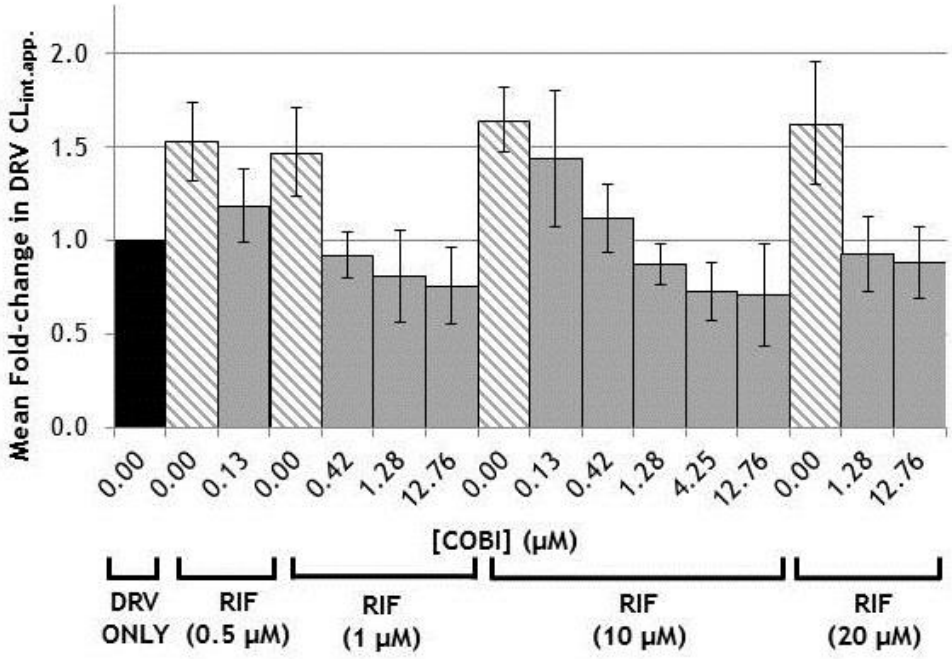

Figure 2: Effects of rifampicin alone, or in combination with cobicistat, on mean DRV $CL_{int.app.}$ in primary human hepatocytes *in vitro.* Cryopreserved primary human hepatocytes were incubated with rifampicin (RIF; 0.5—20 µM), hatched bars; or with cobicistat (COBI; 0.13–12.76 µM) and RIF (0.5—20 µM), grey bars; each day for 72 hours. All cells were then incubated with RIF (0.5—20 µM), or RIF (0.5—20 µM) together with cobicistat (COBI; 0.13–12.76 µM) as described above, together with darunavir (DRV; 5 µM), black bar, for 60 minutes. Control cells were treated with DRV (5 µM) alone for 60 minutes. The results shown represent the mean DRV $CL_{int.app.}$ from three biological replicates measured in hepatocytes from three independent donors (Lot HU1399, HU1574 and HU1587). Error bars: SD.

FIGURE 3

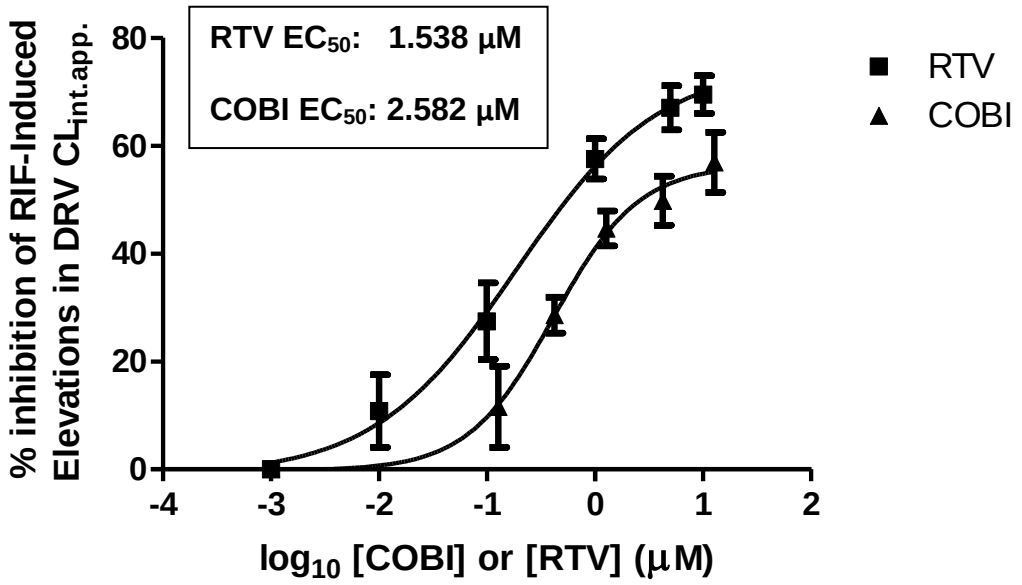

Figure 3: **Relative effectiveness of COBI and RTV at lowering RIF-induced DRV $CL_{int.app.}$.** Line graph shows the percentage inhibition of 10 µM rifampicin (RIF)-induced elevations in DRV $CL_{int.app.}$ in cryopreserved primary human hepatocytes following co-incubation with ritonavir (RTV; 0.1—10 µM; donors HU1399, HU1587 and HU1621), or cobicistat (COBI; 0.13—12.76 µM, donors HU1399, HU1574 and HU1587) in combination with RIF (10 µM) for 72 hours. Each condition was tested in triplicate in each donor. RTV and COBI concentrations are presented as $\log_{10}$ (µM value-0.001). Error bars: SD.

TABLES

TABLE 1

Table 1: Donor Demographic Information for Cryopreserved Primary Human Hepatocytes Used

Donor	Sex	Race	Age	Medications	Drug Use
HU1399	Female	Caucasian	72	Insulin glargine: 10 units *qd*; Metoprolol: 100 mg *qd*; Lisinopril hydrochlorothiazide: 20/12.5 mg *qd*; Calcium + Vitamin D: 500 mg *qd*; Multivitamin: *qd*; Aspirin: 81 mg *qd*	Historic long-term tobacco use
HU1574	Male	Caucasian	70	Atorvastatin: 80 mg *qd*; Lisinopril: 5 mg *qd.*; Aspirin: 81 mg *qd*; Tamsulosin: 4 mg *qd*	None reported
HU1587	Female	Caucasian	43	Vitamin D oral; Multivitamin oral; Calcium + Vitamin D + Vitamin K	None reported
HU1621	Male	Caucasian	66	Pazopanib: 800 mg *qd*	Rare alcohol use. Historic tobacco use