



Published in final edited form as:

HIV Med. 2021 November ; 22(10): 898–906. doi:10.1111/hiv.13136.

***In vitro* assessment of the potential for dolutegravir to affect hepatic clearance of levonorgestrel**

Owain Roberts¹, Hannah Kinvig¹, Andrew Owen², Mohammed Lamorde³, Marco Siccardi¹, Kimberly K. Scarsi⁴

¹Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK

²Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, Materials Innovation Factory, University of Liverpool, Liverpool, UK

³Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda

⁴College of Pharmacy, University of Nebraska Medical Center, Omaha, NE, USA

Abstract

Objectives: The World Health Organization recommends that all countries adopt dolutegravir-based antiretroviral therapy as the preferred regimen for all individuals living with HIV.

Levonorgestrel is a commonly used hormonal contraceptive, which undergoes drug–drug interactions with some antiretrovirals, but the potential interaction between dolutegravir and levonorgestrel has not been examined. We aimed to evaluate cytochrome P450 (CYP)-mediated levonorgestrel metabolism and quantify the effects of dolutegravir on levonorgestrel apparent intrinsic clearance ($CL_{int.app.}$) and *CYP* gene expression.

Methods: *In vitro* CYP-mediated $CL_{int.app.}$ of levonorgestrel was quantified using a recombinant human CYP (rhCYP) enzyme system. A primary human hepatocyte model of drug metabolism was used to assess the effects of dolutegravir on (1) levonorgestrel $CL_{int.app.}$, using liquid chromatography–tandem mass spectrometry, and (2) the expression of specific CYP enzymes, using quantitative real-time polymerase chain reaction.

Results: Levonorgestrel clearance was mediated by multiple rhCYPs, including rhCYP3A4. Under control conditions, levonorgestrel $CL_{int.app.}$ was $22.4 \pm 5.0 \mu\text{L}/\text{min}/10^6$ hepatocytes. Incubation with 43.1 nM of unbound dolutegravir elevated levonorgestrel $CL_{int.app.}$ to 31.4 ± 7.8

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](#) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Correspondence: Marco Siccardi, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, 70 Pembroke Place, Liverpool, L69 3GF, UK. siccardi@liverpool.ac.uk.

^{*}These authors contributed equally to this work.

AUTHOR CONTRIBUTIONS

OR, MS, AO, KKS and HK participated in designing the research. OR and HK conducted experiments. OR, HK and MS performed the data analysis. OR, HK, AO, ML, KKS and MS contributed to the writing of the manuscript.

This study was presented, in part, at the 20th International Workshop on Clinical Pharmacology of HIV, Hepatitis and Other Antiviral Drugs. Noordwijk, The Netherlands. 14–16 May 2019.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

$\mu\text{L}/\text{min}/10^6$ hepatocytes ($P=0.168$), while 142.23 nM increased levonorgestrel $\text{CL}_{\text{int.app.}}$ to $37.0 \pm 2.9 \mu\text{L}/\text{min}/10^6$ hepatocytes ($P=0.012$). Unbound dolutegravir 431 nM induced expression of *CYP3A4* (two-fold) in a dose-dependent manner, while 1.44 μM of unbound dolutegravir induced *CYP2B6* expression 2.2 ± 0.3 -fold ($P=0.0004$).

Conclusions: In summary, this *in vitro* study suggests that dolutegravir has the potential to increase hepatic clearance of levonorgestrel by inducing both CYP3A and non-CYP3A enzymes. The observed *in vitro* dolutegravir–levonorgestrel drug–drug interaction should be further examined in clinical studies.

Keywords

contraceptives; DMPK; drug–drug interactions; pharmacokinetics

INTRODUCTION

The second-generation HIV integrase inhibitor dolutegravir, has a favourable safety profile and a low potential for drug–drug interactions (DDIs) [1]. Nevertheless, a slightly increased risk of neural tube defects occurring in the developing foetuses of women who had received dolutegravir at the time of conception, or during early pregnancy, was reported [2,3].

The World Health Organization guidelines recommend the use of dolutegravir as first-line antiretroviral therapy (ART) in all people living with HIV, with the caveat that effective contraception should also be offered to individuals of childbearing potential, and that they should be fully informed of the potentially increased risk of neural tube defects [4].

Drug–drug interactions are known to occur between some antiretrovirals and hormonal contraceptives [5,6]. For example, we have previously demonstrated a significant DDI between the HIV nonnucleoside reverse transcriptase inhibitor efavirenz and the levonorgestrel contraceptive implant [7]. One study has been conducted with dolutegravir and the ethinyl oestradiol/norgestimate oral contraceptive and found no evidence of DDIs [8]. Two recent studies that evaluated the combination of dolutegravir and another progestin-releasing contraceptive implant – etonogestrel – found a minimal effect in etonogestrel exposure in women receiving dolutegravir [9,10].

There is limited mechanistic information on the pathways involved in the absorption, distribution, metabolism and excretion of steroid hormone contraceptives [11]. For example, oxidative metabolism of levonorgestrel is purported to be mediated by CYP3A4 [12,13], but other mechanisms are also thought to play a role [14]. Levonorgestrel can be administered orally (with high bioavailability) or via subdermal implants; the volume of distribution is estimated to be between 160 and 260 L [15].

The aims of this study were to obtain *in vitro* data to support a better understanding of the interaction between dolutegravir and levonorgestrel by: (1) evaluating the contribution of specific key human cytochrome P450 (CYP) enzymes in levonorgestrel metabolism; (2) quantifying the effect of dolutegravir on the apparent intrinsic clearance ($\text{CL}_{\text{int.app.}}$) of levonorgestrel in a primary human hepatocyte-based *in vitro* model of drug metabolism; (3)

quantifying the potential for dolutegravir to cause DDIs through the expression of key CYP enzymes.

METHODS

A description of the chemicals, reagents, stock solutions and primary hepatocytes used in this study is provided in the supplementary section.

Recombinant human cytochrome P450 enzyme studies and culture of cryopreserved primary human hepatocytes

The incubation method for conducting recombinant human cytochrome P450 (rhCYP) enzyme studies was adapted from Youdim *et al.* [16]. Cryopreserved primary human hepatocytes were cultured as described previously [17]. Following incubation for 60 min, Williams' Medium E (WME) was removed from the cell culture plates and levonorgestrel was extracted and quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described in the supplementary methods section.

Concentrations of drugs used in this study

Primary cryopreserved hepatocytes were incubated with a range of concentrations of dolutegravir, which spanned the unbound concentration range reported in human plasma *in vivo* (10.3–240 ng/mL; 24.6–57.2 nM) [18]. The concentration of levonorgestrel used in hepatocyte experiments (1 nM), was selected as it was within the range of reported plasma concentrations of levonorgestrel released from Jadelle[®] contraceptive subdermal implants between 1 month (1.39 nM; 435 pg/mL) and 3 years (0.90 nM; 280 pg/mL) after implant insertion [19].

The concentration of levonorgestrel used in rhCYP studies (5 μ M), which is several-fold higher than would be encountered *in vivo* [19], was selected to ensure that substrate concentration was not a limiting factor in rhCYP reactions. This concentration was also several-fold lower than the mean observed K_m of progesterone metabolism by CYP3A4, CYP2C19 and CYP2C9 reported in analogous liver microsome studies (80, 37 and 46 μ M, respectively) [20]. The degree of protein binding of dolutegravir in WME incubation medium was determined by a rapid equilibrium dialysis method and is described in the supplementary section [17].

RNA extraction, complementary DNA synthesis and quantitative real-time PCR

Total RNA was extracted from hepatocytes using the Omega BioTek E-Z 96 Total RNA kit (VWR, East Grinstead, UK). RNA quality and quantity were assessed by spectrophotometry using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). First-strand synthesis was carried out using TaqMan[®] Reverse Transcription Reagents (Applied Biosystems, Waltham, MA, USA). Fold-changes in gene expression were determined as described by Pfaffl [21]. Further details are described in the supplementary methods.

Calculation of the apparent intrinsic clearance of levonorgestrel by a single recombinant human CYP

The intrinsic clearance of levonorgestrel achieved by a single rhCYP type – expressed as μL of levonorgestrel cleared/min/pmol of rhCYP ($\text{CL}_{\text{int.app. rhCYP}}$) – was calculated by first calculating the rate of elimination of levonorgestrel by a single rhCYP type in terms of pmol of levonorgestrel eliminated/min/pmol of the rhCYP enzyme in question, and dividing that by the concentration of levonorgestrel (LNG in the equation) present at the start of the incubation (Eq. 1):

$$\text{CL}_{\text{int.app. rhCYP}} = \frac{(\text{pmol of LNG cleared/minute/pmol of rhCYP})}{\text{starting concentration of LNG}} \quad (1)$$

Calculation of the total intrinsic clearance of levonorgestrel by recombinant human CYP

The total intrinsic clearance of levonorgestrel achieved by a single CYP in the liver as a whole (TCL_H) was calculated using Eq. 2, as previously described [22].

$$\text{TCL}_H = \frac{(\text{CL}_{\text{int.app. rhCYP}} \times \text{CYP Abundance} \times \text{Liver Weight} \times \text{MPPGL} \times 1000 \times 60)}{1000000} \quad (2)$$

where rhCYP abundance represents the weighted arithmetic mean abundance of the CYP of interest within in the liver (pmol/mg), as described by Achour *et al.* [23] ; MPPGL is the amount of microsomal protein/g of liver [24] and the liver weight is the arithmetic mean liver weight as described by Molina *et al.* [25]

The total apparent clearance (CL_{app}) of levonorgestrel by hepatic CYPs was calculated as the sum of the intrinsic clearance of each CYP quantified (Eq. 3) [22].

$$\text{CL}_{\text{app}} = \sum \text{TCL}_{\text{int.}} \quad (3)$$

The total systemic plasma clearance (CL) was calculated from levonorgestrel CL_{app} as described in Eq. 4 [22]:

$$\text{CL} = \frac{(Q_{\text{hv}} \times f_u \times \text{CL}_{\text{app}})}{(Q_{\text{hv}} + \text{CL}_{\text{app.}} \times f_u)} \quad (4)$$

where Q_{hv} is the hepatic flow rate and f_u is the fraction of levonorgestrel in plasma that is unbound. As levonorgestrel is reported to be 97% bound to human plasma [26], the levonorgestrel f_u was set as 3%.

Calculation of the $\text{CL}_{\text{int.app.}}$ of levonorgestrel by primary human hepatocytes

The apparent intrinsic clearance ($\text{CL}_{\text{int.app.}}$) of levonorgestrel by primary human hepatocytes was calculated based on a method described by Lu *et al.* [27] (Eq. 5).

$$CL_{\text{int.app.}} = (\ln 2 / \text{in vitro } t_{1/2}) \times (\mu\text{l incubation volume} / 10^6 \text{hepatocytes}). \quad (5)$$

Results were expressed as the arithmetic mean (\pm SD) ($\mu\text{L}/\text{min}/10^6$ hepatocytes) of a total of three donors per condition tested. Three biological replicates were quantified for each condition, tested using cells obtained from three separate donors (HU1574, HU1587 and HU1832; Table 1).

Statistical analysis

Statistical significance was assessed by independent-sample *t*-test with equal variances assumed using IBM® SPSS® Statistics (v.25; IBM Corporation, Armonk, NY, USA), in which *P*-values ≤ 0.05 were considered significant.

RESULTS

Relative contribution of specific recombinant human cytochrome P450 enzymes to levonorgestrel apparent intrinsic clearance *in vitro*

Given that progesterone has been reported to be metabolized by CYP3A4, CYP3A5, CYP2C9 and CYP2C19 [20,28,29], and that levonorgestrel is a progestin-type synthetic steroid hormone contraceptive with a chemical structure similar to that of progesterone, an assessment of potential CYP2C9- and CYP2C19-mediated metabolism of levonorgestrel was warranted, and was tested using a recombinant enzyme system involving the separate incubation of levonorgestrel with baculosomes expressing either rhCYP3A4, or rhCYP3A5, or rhCYP2C9, or rhCYP2C19 following pre-incubation with, or without, selective chemical inhibitors of each respective rhCYP.

Following incubation of levonorgestrel (5 μM) for 60 min, levonorgestrel $CL_{\text{int.app.}}$ mediated by rhCYP3A4 was $0.182 \pm 0.036 \mu\text{L}/\text{min}/\text{pmol}$. Similarly, levonorgestrel $CL_{\text{int.app.}}$ rhCYP3A5 was $0.178 \pm 0.027 \mu\text{L}/\text{min}/\text{pmol}$ following incubation with rhCYP3A5 [30]. Incubation of levonorgestrel with rhCYP2C9 resulted in a levonorgestrel $CL_{\text{int.app.}}$ rhCYP2C9 of $0.062 \pm 0.010 \mu\text{L}/\text{min}/\text{pmol}$ [16,31] while levonorgestrel $CL_{\text{int.app.}}$ rhCYP2C19 was $0.046 \pm 0.005 \mu\text{L}/\text{min}/\text{pmol}$. CYP1A2 and CYP2B6 have important roles in drug metabolism [32] and have also been implicated in steroid hormone metabolism, including the metabolism of testosterone [33] (CYP2B6), oestradiol and oestrone [34] (CYP2B6), and a minor role for CYP1A2 in mediating progesterone metabolism [28]. It was therefore of interest to determine the potential role of rhCYP1A2 and rhCYP2B6 in levonorgestrel metabolism. Levonorgestrel $CL_{\text{int.app.}}$ rhCYP1A2 was $0.028 \pm 0.012 \mu\text{L}/\text{min}/\text{pmol}$ [35]. Levonorgestrel $CL_{\text{int.app.}}$ rhCYP2B6 resulted in a levonorgestrel clearance rate of $0.052 \pm 0.004 \mu\text{L}/\text{min}/\text{pmol}$ [36].

Next, the *in vitro* $CL_{\text{int.app.}}$ rhCYP clearance rates obtained for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and CYP3A5 were used to estimate the total intrinsic clearance of levonorgestrel that would be achieved by each respective single CYP in the adult female liver as a whole (TCL_H) (Eq. 2). The largest TCL_H observed was that of CYP3A4, with a TCL_H of $52.5 \pm 10.4 \text{ L}/\text{h}$ (Figure 1). The TCL_H values of CYP3A5 and CYP2C9 were 9.4 ± 1.4 and $11.6 \pm 1.9 \text{ L}/\text{h}$, respectively (Figure 1). By comparison, the

relative contributions of CYP1A2, CYP2B6 and CYP2C19 to levonorgestrel TCL_H were 3.4 ± 1.5 , 2.6 ± 0.2 and 1.6 ± 0.2 L/h, respectively (Figure 1).

The total apparent clearance (CL_{app}) of levonorgestrel by the hepatic CYPs measured was 80.97 ± 15.53 L/h, and the total systemic plasma clearance of levonorgestrel was 2.38 ± 0.46 L/h.

Effects of dolutegravir on the apparent intrinsic clearance of levonorgestrel

The use of cryopreserved human hepatocytes is a recommended method for assessing metabolism-based DDIs *in vitro* [37]. As such, the effect of dolutegravir on levonorgestrel $CL_{int.app.}$ by cryopreserved primary human hepatocytes obtained from three different donors (HU1574, HU1587 and HU1832) was quantified. Under control conditions, where hepatocytes were incubated with 1 nM levonorgestrel alone, levonorgestrel $CL_{int.app.}$ was 22.4 ± 5.0 $\mu\text{L}/\text{min}/10^6$ cells. Rifampin was used as a positive control to induce levonorgestrel $CL_{int.app.}$ by hepatocytes. Incubation with 10 μM rifampin has previously been shown to optimally induce CYP3A4 enzymatic activity in hepatocytes *in vitro* [38,39], including achieving the maximal $CL_{int.app.}$ of CYP3A4 substrates in this model [17]. As expected, incubation with 10 μM rifampin for 72 h increased levonorgestrel $CL_{int.app.}$ by 109%, to 46.7 ± 6.9 $\mu\text{L}/\text{min}/10^6$ cells ($P = 0.008$; Figure 2).

The median unbound dolutegravir ($DTG_{unbound}$) reported in human plasma is 24.6–57.2 nM [18]. We selected concentrations of $DTG_{unbound}$ to span this range when assessing the effects of dolutegravir on levonorgestrel $CL_{int.app.}$. Quantification of the amount of protein-binding of dolutegravir within our hepatocyte-based model of drug metabolism showed that the unbound fraction (f_u) of dolutegravir in WME incubation medium was 0.43 ± 0.09 ($n = 9$). A levonorgestrel $CL_{int.app.}$ of 34.5 ± 9.2 $\mu\text{L}/\text{min}/10^6$ cells ($P = 0.114$; Figure 2) was observed following 72 h incubation with 0.033 μM dolutegravir ($DTG_{unbound} = 14.2$ nM). A trend towards elevated levonorgestrel $CL_{int.app.}$ was apparent at all concentrations of dolutegravir tested, with 0.1 μM dolutegravir ($DTG_{unbound} = 43.1$ nM), resulting in a levonorgestrel $CL_{int.app.}$ of 31.4 ± 7.8 $\mu\text{L}/\text{min}/10^6$ cells ($P = 0.168$; Figure 2). Incubation of hepatocytes with 0.33 μM dolutegravir ($DTG_{unbound} = 142.23$ nM) and 3.33 μM dolutegravir ($DTG_{unbound} = 1.44$ μM) for 72 h significantly increased levonorgestrel $CL_{int.app.}$ to 37.0 ± 2.9 $\mu\text{L}/\text{min}/10^6$ cells ($P = 0.012$), and 32.8 ± 4.2 $\mu\text{L}/\text{min}/10^6$ cells ($P = 0.050$), respectively (Figure 2).

Analysis of the effects of dolutegravir on the expression of CYP1A2, CYP2B6 and CYP3A4 in human hepatocytes

United States Food and Drug Administration guidelines recommend evaluation of the potential of an investigational drug to induce *CYP1A2*, *CYP2B6* and *CYP3A4* [37]. To evaluate the potential of dolutegravir to induce CYP expression, an *in vitro* gene expression assay in human hepatocytes was used to assess the effects of incubating a range of concentrations of dolutegravir (0.00033–10 μM ; $DTG_{unbound}$, 0.142–4310 nM) on the expression of *CYP1A2*, *CYP2B6* and *CYP3A4*. Also, hepatocytes were incubated separately with phenobarbital (1 mM) – a positive control for induction of the expression of

CYP1A2 [40] and *CYP2B6* [41] – as well as rifampin (10 μM), which served as a positive control for the induction of the expression of *CYP2B6* [41], and *CYP3A4* [38,42].

Although incubation with the *CYP1A2* inducer phenobarbital (1 mM) induced *CYP1A2* expression by 2.7 ± 0.6 -fold ($P = 0.043$; Figure 3a), dolutegravir did not induce the expression of *CYP1A2* (by two-fold above vehicle control) at any of the concentrations tested (Figure 3a). By contrast, incubation with a total dolutegravir concentration of 3.33 μM ($\text{DTG}_{\text{unbound}} = 1.44 \mu\text{M}$) and 10 μM ($\text{DTG}_{\text{unbound}} = 4.310 \mu\text{M}$) induced the expression of *CYP2B6* by 2.2 ± 0.3 -fold and 2.3 ± 0.7 -fold, respectively (Figure 3b). Under the same conditions, phenobarbital (1 mM) induced *CYP2B6* expression by 2.6 ± 1.1 -fold (Figure 3b), while rifampin (10 μM) induced *CYP2B6* expression by 2.0 ± 0.5 -fold (Figure 3b).

Incubation with rifampin (10 μM) induced a 7.7 ± 1.9 -fold increase in *CYP3A4* expression, while incubation with phenobarbital (1 mM) elevated *CYP3A4* expression levels 4.8 ± 0.7 -fold (Figure 3c). Incubation with $\text{DTG}_{\text{unbound}} = 431 \text{ nM}$ (total dolutegravir concentration of 1 μM), significantly induced expression of *CYP3A4* by 2.3 ± 0.4 -fold (Figure 3c). Dolutegravir-induced expression of *CYP3A4* increased in a dose-dependent manner, with 3.33 μM dolutegravir ($\text{DTG}_{\text{unbound}} = 1.44 \mu\text{M}$) resulting in a 3.5 ± 0.7 -fold induction of *CYP3A4* expression, and 10 μM dolutegravir ($\text{DTG}_{\text{unbound}} = 4.31 \mu\text{M}$) eliciting a 4.1 ± 1.0 -fold induction (Figure 3c).

DISCUSSION

The need to assess the likelihood of DDIs occurring between antiretrovirals and hormonal contraceptives has recently been highlighted [43]. However, the mechanistic potential for DDIs between dolutegravir and levonorgestrel had not been previously examined. Furthermore, a paucity of information existed regarding the metabolic pathways mediating levonorgestrel metabolism. Here, it was found that rhCYP3A4 was the predominant CYP mediating apparent clearance of levonorgestrel (Figure 1), a finding that supports the established view that CYP3A4 is an important mediator of levonorgestrel metabolism [12,13]. Interestingly, secondary to CYP3A4, both CYP2C9 and CYP3A5 mediated apparent clearance of levonorgestrel to a similar degree (Figure 1). Meanwhile, CYP1A2, CYP2B6 and CYP2C19 each had relatively minor roles as mediators of levonorgestrel clearance (Figure 1).

It was recently concluded that CYP3A may not represent the predominant pathway by which levonorgestrel is metabolized [11]. A recently published study examining the effects of rifampin on the pharmacokinetics of various progestin-based oral contraceptives showed that exogenous progestins exhibited differential vulnerability to rifampin-mediated CYP3A4 induction [14], and that levonorgestrel was among the least vulnerable progestins to rifampin-induced CYP3A4 activity. The present study supports this observation, showing a predominant, but not exclusive, role for CYP3A4 in mediating levonorgestrel metabolism [14]. CYP3A4 is the predominant CYP involved in progesterone metabolism [20,28,29], and our findings reveal that the CYPs involved in metabolism of levonorgestrel share some similarity with CYP-mediated progesterone metabolism pathways, where CYP1A2 [28], CYP2C9 [20,28] and CYP2C19 [20,28] each has a contributory role, secondary to CYP3A4.

It remains possible, therefore, that other CYPs may also contribute to levonorgestrel metabolism. Indeed, a role for CYP3A7 – the predominant foetal isoform of CYP3A enzymes – as an important mediator of etonogestrel metabolism, was recently described [44]. Levonorgestrel implants are contraindicated in women receiving antiepileptic drugs due to the potential for DDIs that may result in contraceptive failure [45]. Notwithstanding the CYP3A induction potential of the antiepileptic drugs phenytoin and oxcarbazepine, phenytoin–levonorgestrel and oxcarbazepine–levonorgestrel DDIs may be mediated by non-CYP3A enzymes [46,47]. Indeed, this further underlines the recently reviewed general need to better define the pharmacokinetics of mixed-mechanism DDIs [48]. While administering CYP3A inducers can decrease systemic levonorgestrel exposure by > 40% *in vivo* [11], it has been highlighted that the CYP3A inducers used when conducting these studies also have the potential to induce the expression of other enzymes, including phase II enzymes, together with multiple phase I enzymes, and drug transporters [11]. Although assessing the relative contribution of phase II enzymes and drug transporters in levonorgestrel clearance was beyond the scope of the current study, this should be addressed in future *in vitro* studies in order to better evaluate the DDI vulnerability of levonorgestrel.

The total systemic plasma clearance of levonorgestrel was estimated to be 2.38 ± 0.46 L/h. The total clearance of levonorgestrel by various routes of administration in adult females ranges between 3.9 and 4.2 L/h [15,49]. The reasons for these differential values may be explained by the following: (1) the potential contribution of CYPs other than rhCYP1A2, rhCYP2B6, rhCYP2C9, rhCYP2C19, rhCYP3A4 and rhCYP3A5 were not quantified in the present study; (2) the potential contribution of phase II enzymes and drug transporters were not considered when estimating levonorgestrel total systemic plasma clearance using rhCYP data herein; (3) the potential contribution of extrahepatic metabolism of levonorgestrel was not considered; (4) the likely effects of interindividual variability in CYP expression and other pharmacokinetic parameters were not considered; (5) *in vitro* rhCYP systems are known to frequently underestimate drug clearance and may differ from the individuals included in the reported studies; and (6) the integration of protein binding in our calculation could differ from the clinical studies used for the comparison [50,51].

The potential for dolutegravir to cause a DDI was measured by quantifying the effects of dolutegravir on the expression of *CYP1A2*, *CYP2B6* and *CYP3A4* [52]. At unbound dolutegravir concentrations 7.5-fold higher than have been reported in plasma, dolutegravir induced the expression of *CYP2B6* and *CYP3A4*, but did not induce increased expression of *CYP1A2* (Figure 3). The observed effects of dolutegravir on gene expression contradict a previous study [53], which suggested that expression of neither *CYP2B6* nor *CYP3A4* was affected at total dolutegravir concentrations up to and including 40 μ M [53]. The reasons for these differences are not known, but may be due to inter-donor variation in the hepatocytes, or to differences in the duration of incubation with dolutegravir with hepatocytes (48 h [53] vs. 72 h in the present study). Indeed, while incubation of hepatocytes with test compounds for either 48 or 72 h are both recommended for *in vitro* assessment of the induction potential of compounds [52], consideration should be given to the fact that chronically administered drugs, including dolutegravir, will be administered *in vivo* for prolonged periods of time. Furthermore, studies using prototypic inducers of CYP expression have shown that temporal differences can lead to differential expression of key

drug-metabolizing CYPs [41,54,55]. Nevertheless, at concentrations spanning the range of unbound concentrations of dolutegravir reported in human plasma (10.3–240 ng/mL; 24.6–57.2 nM [18]) dolutegravir had no effect on the expression of *CYP2B6* or *CYP3A4* in the present study (Figure 3). *CYP2C9* is characterized by lower (two-to three-fold), but correlated, inducibility compared with *CYP3A4*, and, consequently, it is likely that at relevant dolutegravir concentrations *CYP2C9* does not play a significant role in this interaction [56].

In vitro data was used to describe the combination of dolutegravir and levonorgestrel implants by quantifying the effects of dolutegravir on levonorgestrel $CL_{int.app.}$. Using an *in vitro* hepatocyte-based model of drug metabolism [17], the incubation with rifampin increased levonorgestrel clearance by 109%, and this experimental effect is comparable to clinical observation, where levonorgestrel exposure was decreased by 58% [14]. Incubation with dolutegravir at concentrations spanning the range of median $DTG_{unbound}$ in plasma (10.3–240 ng/mL; 24.6–57.2 nM) [18] resulted in a trend towards elevated levonorgestrel $CL_{int.app.}$ (Figure 2). Therefore, while these observations show that a DDI exists between dolutegravir and levonorgestrel *in vitro*, it remains unclear whether a dolutegravir–levonorgestrel DDI may occur with the $DTG_{unbound}$ plasma concentrations encountered *in vivo*.

In summary, this study reveals novel CYP-mediated mechanisms of levonorgestrel metabolism, supporting the notion that levonorgestrel undergoes metabolism by CYP enzymes additional to CYP3A, and shows that incubation with dolutegravir can significantly elevate levonorgestrel $CL_{int.app.}$ and expression of *CYP2B6* and *CYP3A4*, albeit at concentrations greater than the clinical therapeutic range of dolutegravir in humans, raising the possibility that clinical dolutegravir–levonorgestrel DDIs may occur. Future studies should aim to further elucidate the mechanisms mediating levonorgestrel metabolism *in vitro*, and clinical studies should aim to evaluate the effects of dolutegravir on levonorgestrel exposure in humans to ensure their safe and effective combination for females living with HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding information

This study was funded by the National Institutes of Health (NIH), Eunice Kennedy Shriver National Institute of Child Health and Human Development [Grant Number: 1R01HD085887 (Scarsi)]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

REFERENCES

1. Cottrell ML, Hadzic T, Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. *Clin Pharmacokinet* 2013; 52: 981–994. [PubMed: 23824675]
2. Zash R, Makhema J, Shapiro RL. Neural-tube defects with dolutegravir treatment from the time of conception. *N Engl J Med* 2018; 379: 979–981. [PubMed: 30037297]

3. World Health Organisation. Potential safety issue affecting women living with HIV using dolutegravir at the time of conception. Geneva, Switzerland, 2018.
4. World Health Organisation. Update of recommendations on first-and second-line antiretroviral regimens. Geneva, Switzerland, 2019.
5. Robinson JA, Jamshidi R, Burke AE. Contraception for the HIV-positive woman: a review of interactions between hormonal contraception and antiretroviral therapy. *Infect Dis Obstet Gynecol.* 2012; 2012: 890160. [PubMed: 22927715]
6. Nanda K, Stuart GS, Robinson J, Gray AL, Tepper NK, Gaffield ME. Drug interactions between hormonal contraceptives and antiretrovirals. *AIDS* 2017; 31: 917–952. [PubMed: 28060009]
7. Scarsi KK, Darin KM, Nakalema S, et al. Unintended pregnancies observed with combined use of the levonorgestrel contraceptive implant and efavirenz-based antiretroviral therapy: a three-arm pharmacokinetic evaluation over 48 weeks. *Clin Infect Dis* 2016; 62: 675–682. [PubMed: 26646680]
8. Song IH, Borland J, Chen S, Wajima T, Peppercorn AF, Piscitelli SC. Dolutegravir has no effect on the pharmacokinetics of oral contraceptives with norgestimate and ethinyl estradiol. *Ann Pharmacother* 2015; 49: 784–789. [PubMed: 25862012]
9. Bishop IJ, Gertz AM, Simon B, et al. Etonogestrel concentrations among contraceptive implant users in Botswana using and not using dolutegravir-based antiretroviral therapy. *Contraception* 2020; 102: 174–179. [PubMed: 32387328]
10. Patel R, Stalter R, Onono M, et al. Dolutegravir-containing ART does not reduce etonogestrel implant concentrations. Conference on Retroviruses and Opportunistic Infections. Boston, MA, 2020.
11. Zhang N, Shon J, Kim MJ, et al. Role of CYP3A in oral contraceptives clearance. *Clin Transl Sci* 2018; 11: 251–260. [PubMed: 28986954]
12. Bayer New Zealand Limited. Jadelle[®], Subcutaneous Implants, Levonorgestrel 2 × 75 mg, Data Sheet. 2015. <http://www.medsafe.govt.nz/Profs/Datasheet/j/Jadelleimplant.pdf>. Accessed 9 November 2020.
13. Food and Drug Administration. Highlights of Prescribing Information -JADELLE (levonorgestrel implants) for subdermal use. 2016. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/020544s010lbl.pdf. Accessed 9 November 2020.
14. Wiesinger H, Klein S, Rottmann A, et al. The effects of weak and strong CYP3A induction by rifampicin on the pharmacokinetics of five progestins and ethinylestradiol compared to midazolam. *Clin Pharmacol Ther* 2020; 108: 798–807. [PubMed: 32275771]
15. Kook K, Gabelnick H, Duncan G. Pharmacokinetics of levonorgestrel 0.75 mg tablets. *Contraception* 2002; 66: 73–76. [PubMed: 12169384]
16. Youdim KA, Zayed A, Dickins M, et al. Application of CYP3A4 in vitro data to predict clinical drug-drug interactions; predictions of compounds as objects of interaction. *Br J Clin Pharmacol* 2008; 65: 680–692. [PubMed: 18279465]
17. Roberts O, Khoo S, Owen A, Siccardi M. Interaction of rifampin and darunavir-ritonavir or darunavir-cobicistat in vitro. *Antimicrob Agents Chemother* 2017; 61.
18. Food and Drug Administration. Dolutegravir, GSK1349572, Clinical Pharmacology Review. 2012. <https://www.fda.gov/downloads/Drugs/.../UCM370760.pdf>. Accessed 04 April 2019.
19. Sivin I, Wan L, Ranta S, et al. Levonorgestrel concentrations during 7 years of continuous use of Jadelle contraceptive implants. *Contraception* 2001; 64: 43–49. [PubMed: 11535213]
20. Yamazaki H, Shimada T. Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9, and 3A4 in human liver microsomes. *Arch Biochem Biophys* 1997; 346: 161–169. [PubMed: 9328296]
21. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; 29: e45. [PubMed: 11328886]
22. Rajoli RK, Back DJ, Rannard S, et al. Physiologically based pharmacokinetic modelling to inform development of intramuscular long-acting nanoformulations for HIV. *Clin Pharmacokinet* 2015; 54: 639–650. [PubMed: 25523214]

23. Achour B, Barber J, Rostami-Hodjegan A. Expression of hepatic drug-metabolizing cytochrome p450 enzymes and their intercorrelations: a meta-analysis. *Drug Metab Dispos* 2014; 42: 1349–1356. [PubMed: 24879845]
24. Barter ZE, Chowdry JE, Harlow JR, Snawder JE, Lipscomb JC, Rostami-Hodjegan A. Covariation of human microsomal protein per gram of liver with age: absence of influence of operator and sample storage may justify interlaboratory data pooling. *Drug Metab Dispos* 2008; 36: 2405–2409. [PubMed: 18775982]
25. Molina DK, DiMaio VJ. Normal organ weights in women: part ii-the brain, lungs, liver, spleen, and kidneys. *Am J Forensic Med Pathol* 2015; 36: 182–187. [PubMed: 26108038]
26. Qi-Gui L, Hümpel M. Serum protein binding characteristics of cyproterone acetate, gestodene, levonorgestrel and norethisterone in rat, rabbit, dog, monkey and man. *J Steroid Biochem* 1990; 35: 319–326. [PubMed: 2137890]
27. Lu C, Li P, Gallegos R, et al. Comparison of intrinsic clearance in liver microsomes and hepatocytes from rats and humans: evaluation of free fraction and uptake in hepatocytes. *Drug Metab Dispos* 2006; 34: 1600–1605. [PubMed: 16790553]
28. Niwa T, Yabusaki Y, Honma K, et al. Contribution of human hepatic cytochrome P450 isoforms to regioselective hydroxylation of steroid hormones. *Xenobiotica* 1998; 28: 539–547. [PubMed: 9667077]
29. Waxman DJ, Lapenson DP, Aoyama T, Gelboin HV, Gonzalez FJ, Korzekwa K. Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome P450s. *Arch Biochem Biophys* 1991; 290: 160–166. [PubMed: 1898086]
30. Stresser DM, Broudy MI, Ho T, et al. Highly selective inhibition of human CYP3Aa in vitro by azamulin and evidence that inhibition is irreversible. *Drug Metab Dispos* 2004; 32: 105–112. [PubMed: 14709627]
31. Bourrie M, Meunier V, Berger Y, Fabre G. Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. *J Pharmacol Exp Ther* 1996; 277: 321–332. [PubMed: 8613937]
32. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013; 138: 103–141. [PubMed: 23333322]
33. Rendic S, Nolteernsting E, Schanzer W. Metabolism of anabolic steroids by recombinant human cytochrome P450 enzymes. Gas chromatographic-mass spectrometric determination of metabolites. *J Chromatogr B Biomed Sci Appl* 1999; 735: 73–83. [PubMed: 10630892]
34. Yamazaki H, Shaw PM, Guengerich FP, Shimada T. Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. *Chem Res Toxicol* 1998; 11: 659–665. [PubMed: 9625734]
35. Cho US, Park EY, Dong MS, Park BS, Kim K, Kim KH. Tight-binding inhibition by alpha-naphthoflavone of human cytochrome P450 1A2. *Biochim Biophys Acta* 2003; 1648: 195–202. [PubMed: 12758162]
36. Melis V, Usach I, Gandia P, Peris JE. Inhibition of efavirenz metabolism by sertraline and nortriptyline and their effect on efavirenz plasma concentrations. *Antimicrob Agents Chemother* 2016; 60: 1022–1028. [PubMed: 26643342]
37. Food and Drug Administration. In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry. 2020. <https://www.fda.gov/media/134582/download>. Accessed 9 November 2020.
38. Fahmi OA, Kish M, Boldt S, Obach RS. Cytochrome P450 3A4 mRNA is a more reliable marker than CYP3A4 activity for detecting pregnane X receptor-activated induction of drug-metabolizing enzymes. *Drug Metab Dispos* 2010; 38: 1605–1611. [PubMed: 20566695]
39. Hariparsad N, Nallani SC, Sane RS, Buckley DJ, Buckley AR, Desai PB. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *J Clin Pharmacol* 2004; 44: 1273–1281. [PubMed: 15496645]
40. Yoshinari K, Yoda N, Toriyabe T, Yamazoe Y. Constitutive androstane receptor transcriptionally activates human CYP1A1 and CYP1A2 genes through a common regulatory element in the 5'-flanking region. *Biochem Pharmacol* 2010; 79: 261–269. [PubMed: 19682433]

41. Faucette SR, Wang H, Hamilton GA, et al. Regulation of CYP2B6 in primary human hepatocytes by prototypical inducers. *Drug Metab Dispos* 2004; 32: 348–358. [PubMed: 14977870]
42. Bjornsson TD, Callaghan JT, Einolf HJ, et al. The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* 2003; 31: 815–832. [PubMed: 12814957]
43. Ahn MR, Li L, Shon J, Bashaw ED, Kim MJ. Teratogenic drugs and their drug interactions with hormonal contraceptives. *Clin Pharmacol Ther* 2016; 100: 217–219. [PubMed: 27090193]
44. Lazorwitz A, Aquilante CL, Oreschak K, Sheeder J, Guiahi M, Teal S. Influence of genetic variants on steady-state etonogestrel concentrations among contraceptive implant users. *Obstet Gynecol* 2019; 133: 783–794. [PubMed: 30870275]
45. Crawford P Interactions between antiepileptic drugs and hormonal contraception. *CNS Drugs* 2002; 16: 263–272. [PubMed: 11945109]
46. Crawford P, Chadwick DJ, Martin C, Tjia J, Back DJ, Orme M. The interaction of phenytoin and carbamazepine with combined oral contraceptive steroids. *Br J Clin Pharmacol* 1990; 30: 892–896. [PubMed: 2126946]
47. Fattore C, Cipolla G, Gatti G, et al. Induction of ethinylestradiol and levonorgestrel metabolism by oxcarbazepine in healthy women. *Epilepsia* 1999; 40: 783–787. [PubMed: 10368079]
48. Ramsden D, Fung C, Hariparsad N, et al. Perspectives from the innovation and quality consortium induction working group on factors impacting clinical drug-drug interactions resulting from induction: focus on cytochrome 3A substrates. *Drug Metab Dispos* 2019; 47: 1206–1221. [PubMed: 31439574]
49. Reinecke I, Hofmann B, Mesic E, Drenth HJ, Garmann D. An Integrated population pharmacokinetic analysis to characterize levonorgestrel pharmacokinetics after different administration routes. *J Clin Pharmacol* 2018; 58: 1639–1654. [PubMed: 30207604]
50. Stringer RA, Strain-Damerell C, Nicklin P, Houston JB. Evaluation of recombinant cytochrome P450 enzymes as an in vitro system for metabolic clearance predictions. *Drug Metab Dispos* 2009; 37: 1025–1034. [PubMed: 19196847]
51. Galetin A, Brown C, Hallifax D, Ito K, Houston JB. Utility of recombinant enzyme kinetics in prediction of human clearance: impact of variability, CYP3A5, and CYP2C19 on CYP3A4 probe substrates. *Drug Metab Dispos* 2004; 32: 1411–1420. [PubMed: 15342470]
52. Food and Drug Administration. Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. 2017. <https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table1>. Accessed 9 November 2020.
53. Reese MJ, Savina PM, Generaux GT, et al. In vitro investigations into the roles of drug transporters and metabolizing enzymes in the disposition and drug interactions of dolutegravir, a HIV integrase inhibitor. *Drug Metab Dispos* 2013; 41: 353–361. [PubMed: 23132334]
54. Zhang JG, Ho T, Callendrello AL, Crespi CL, Stresser DM. A multi-endpoint evaluation of cytochrome P450 1A2, 2B6 and 3A4 induction response in human hepatocyte cultures after treatment with beta-naphthoflavone, phenobarbital and rifampicin. *Drug Metab Lett* 2010; 4: 185–194. [PubMed: 20583967]
55. LeCluyse E, Madan A, Hamilton G, Carroll K, DeHaan R, Parkinson A. Expression and regulation of cytochrome P450 enzymes in primary cultures of human hepatocytes. *J Biochem Mol Toxicol* 2000; 14: 177–188. [PubMed: 10789495]
56. Endo-Tsukude C, Kato M, Kaneko A, et al. Risk of CYP2C9 induction analyzed by a relative factor approach with human hepatocytes. *Drug Metab Pharmacokinet* 2019; 34: 325–333. [PubMed: 31564409]

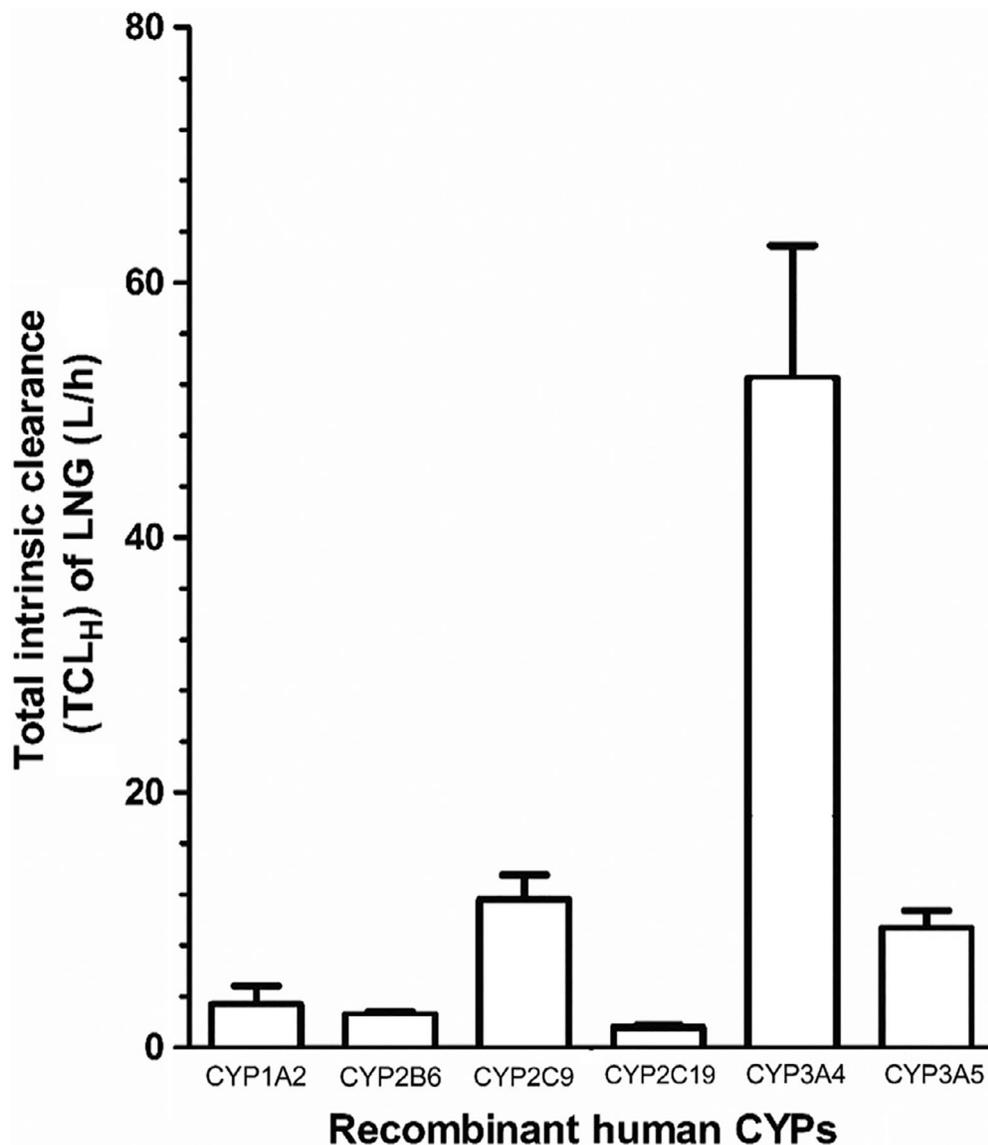


FIGURE 1.

Predicted contribution of cytochrome P450 (CYP) enzymes to the total intrinsic clearance of levonorgestrel in the female liver. Baculosomes[®] expressing recombinant human cytochrome P450 (rhCYP) enzymes (rhCYP1A2, rhCYP2B6, rhCYP2C9, rhCYP2C19, rhCYP3A4 or rhCYP3A5) were incubated for 60 min at 37°C with levonorgestrel (LNG; 5 μM) alone (test samples), or were pre-incubated for 15 min each at 37°C with the following CYP inhibitors – α-naphthoflavone (3 μM; CYP1A2 inhibitor); sertraline (16 μM; CYP2B6 inhibitor); sulfaphenazole (10 μM; CYP2C9 inhibitor); (+)-N-3-benzylnirvanol (10 μM; CYP2C19 inhibitor); ketoconazole (10 μM; CYP3A4 inhibitor); or ketoconazole (30 μM; CYP3A5 inhibitor) – prior to incubation with levonorgestrel (5 μM) at 37°C for 60 min (inhibitor control samples). The amount of levonorgestrel present following incubation with levonorgestrel for 60 min was quantified by high-performance liquid chromatography-UV at 230 nm. Levonorgestrel clearance by individual rhCYPs was calculated by comparing

levonorgestrel concentrations in test samples with those of respective inhibitor control samples. The obtained clearance values were subsequently scaled up as described in the text (Eq. 2) [22] to predict the total intrinsic clearance of levonorgestrel achieved by a single CYP in the female liver as a whole (TCL_H). The values shown are the arithmetic mean of three independent experiments performed in triplicate. Error bars represent SD

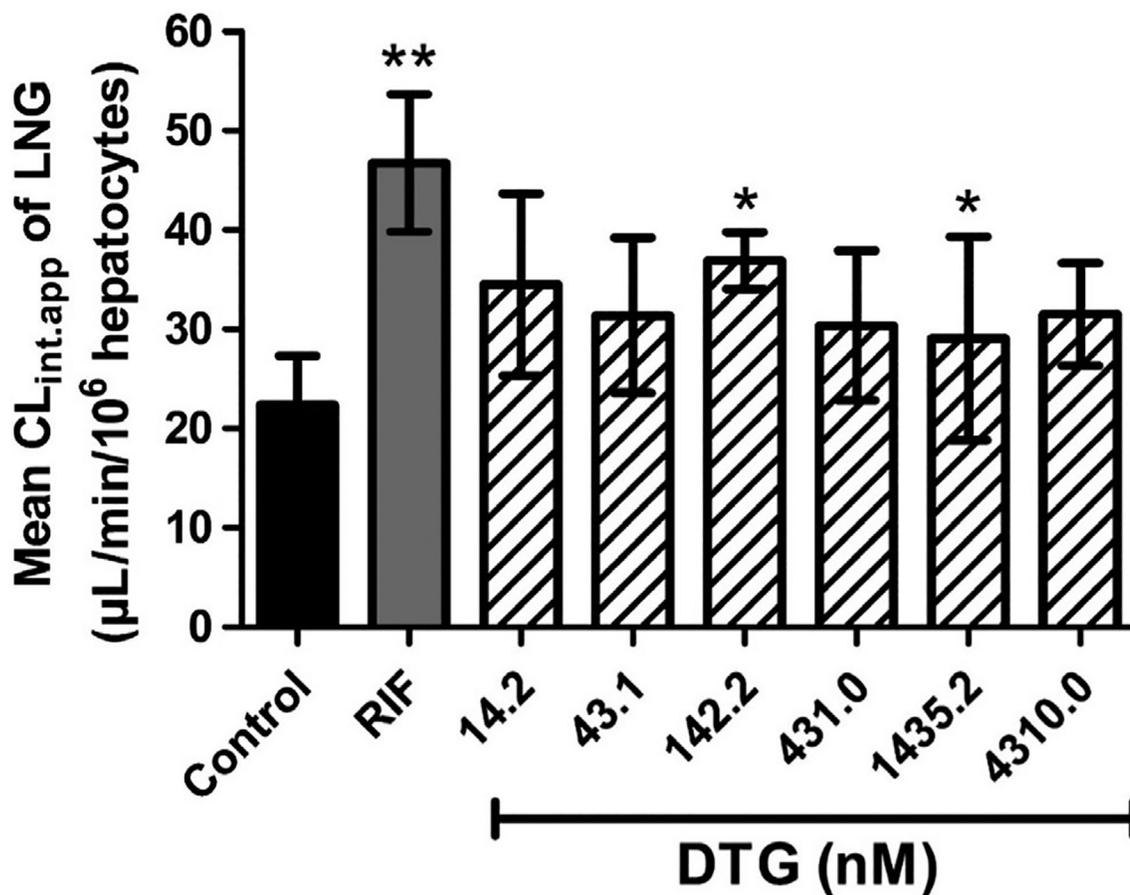
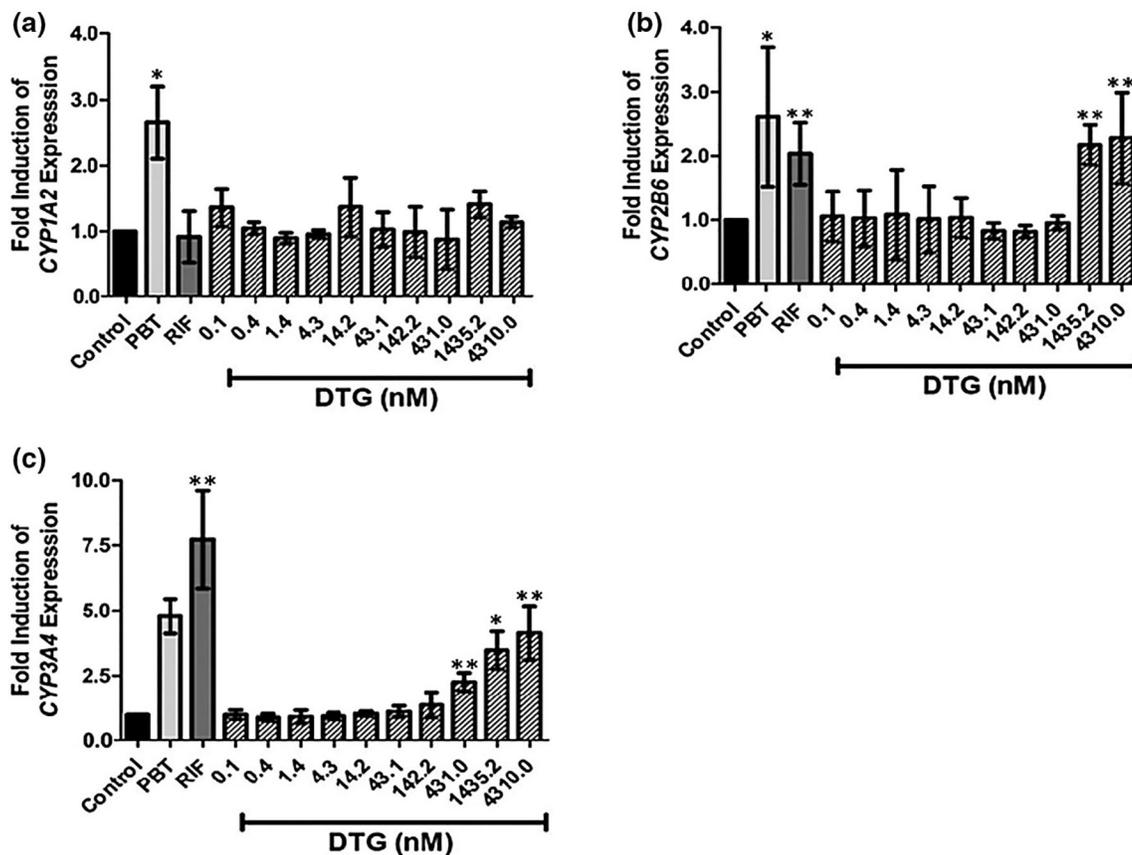


FIGURE 2.

Effects of dolutegravir on the apparent intrinsic clearance ($CL_{int.app.}$) of levonorgestrel by cryopreserved primary human hepatocytes. Cryopreserved primary human hepatocytes from three different donors (HU1574, HU1587 and HU1832) were incubated separately with 10 μM rifampin (RIF; positive control; grey bar), or with a range of concentrations of dolutegravir (DTG; 0.033–10 μM ; unbound DTG concentrations, 14.2–4310 nM; hatched bars) for 72 h prior to incubation with levonorgestrel (1 nM) for 60 min. The mean levonorgestrel $CL_{int.app.}$ by hepatocytes incubated with levonorgestrel only is also shown (control; black bar). The results shown represent the mean levonorgestrel $CL_{int.app.}$ of three biological replicates, using hepatocytes obtained from three separate donors. The concentrations of DTG shown are those of unbound DTG concentrations in Williams' Medium E following correction for protein binding. Error bars = SD. * P 0.05 vs. control; ** P 0.01 vs. control (unpaired t -test)

**FIGURE 3.**

Effects of dolutegravir (DTG) on cytochrome (CYP) P450 expression in human hepatocytes. Mean fold-induction of expression of *CYP1A2* (a), *CYP2B6* (b), *CYP3A4* (c) in human hepatocytes is shown. Cryopreserved primary human hepatocytes from donors HU1587 and HU1832 were incubated separately with 1 mM phenobarbital (PBT; light grey bar), 10 μ M rifampin (RIF; dark grey bar), or with a range of concentrations of DTG (0.0033–10 μ M; unbound DTG concentrations, 0.1–4310 nM; hatched bars) for 72 h prior to extraction of total RNA and subsequent cDNA synthesis and quantitative real-time PCR. The results shown represent the arithmetic mean fold-change in gene expression relative to the gene expression following incubation with vehicle control (0.3% v/v methanol) for 72 h, which was set at 1.0 in each case. Each condition was tested in triplicate using hepatocytes obtained from two separate donors. The concentrations of DTG shown are those of unbound DTG concentrations in Williams' Medium E following correction for protein binding. Error bars = SD. * P < 0.05 vs. control; ** P < 0.01 vs. control (unpaired t -test)

TABLE 1

Donor demographic and medication information for cryopreserved primary human hepatocytes used

Donor	Sex	Race	Age (years)	Medications	Drug use
HUI1574	Male	Caucasian	70	Atorvastatin, 80 mg q.d.; lisinopril, 5 mg q.d.; aspirin, 81 mg q.d.; Tamsulosin, 4 mg q.d.	None reported
HUI1587	Female	Caucasian	43	Vitamin D oral; multivitamin oral; calcium + vitamin D + vitamin K	None reported
HUI1832	Female	Caucasian	53	None reported	Alcohol and tobacco use

Note: q.d., once a day.