Inhibitory Effects of Selected Antituberculosis Drugs on Common Human Hepatic Cytochrome P450 and UDP-glucuronosyltransferase Enzymes[§]

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ABSTRACT

The comorbidities of tuberculosis and diseases such as HIV require long-term treatment with multiple medications. Despite substantial *in vitro* and *in vivo* information on effects of rifampicin and isoniazid on human CYPs, there is limited published data regarding the inhibitory effects of other anti-TB drugs on human CYPs and UGTs. The inhibitory effects of five first-line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and rifabutin), and the newly approved bedaquiline, were evaluated for six common human hepatic UGT enzymes (UGT1A1, 1A4, 1A6, 1A9, 2B7 and 2B15) *in vitro* using HLMs. Pyrazinamide, ethambutol, rifabutin and

bedaquiline were also studied for their inhibitory effects on eight of the most common human CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A). Rifabutin inhibited multiple CYPs to varying degrees *in vitro*, but with all IC₅₀ values exceeding 25 μ M. Rifabutin and rifampicin also inhibited several human UGTs including UGT1A4. The K_i value for rifabutin on human hepatic UGT1A4 was 2 μ M. Finally, the six anti-TB drugs produced minimal inhibition of acetaminophen glucuronidation *in vitro*. Overall, the findings do not raise major concerns regarding metabolic inhibition of human hepatic CYPs and UGTs by the tested anti-TB drugs.

Introduction

Tuberculosis is one of the leading causes of morbidity and mortality worldwide. The World Health Organization estimated that in 2015 there were 10.4 million incident TB cases, and 1.4 million deaths from TB, and an additional 0.4 million deaths associated with co-infection with HIV (World Health Organization (WHO), 2016). The comorbidity of TB and other diseases requires treatment with multiple medications. Understanding of potential drug-drug interactions (DDIs) is of importance in planning safe and effective combination therapies.

Isoniazid, rifampicin (or rifampin), pyrazinamide, ethambutol, rifabutin, and rifapentine are the principal first-line anti-TB drugs to treat drug-susceptible tuberculosis (Zumla et al., 2013). Bedaquiline is a novel antimycobacterial agent which was approved by FDA in 2012 to treat multidrug resistant tuberculosis (Worley and Estrada, 2014). Among those, rifampicin is a potent inducer of CYPs and UGTs, as well as the P-glycoprotein transport system both *in vitro* (Rae et al., 2001; Soars et al., 2004) and clinically (Baciewicz et al., 2013). Rifampicin is reported also to

be an inhibitor of some human CYPs *in vitro* (Kajosaari et al., 2005), but its overall effect is enzymatic induction, reducing systemic concentrations of many drugs (Ochs et al., 1981). Compared with rifampicin, rifabutin has less potency as a CYP3A inducer and is used as a substitute for rifampicin in patients receiving protease inhibitor and integrase inhibitor-based antiretroviral therapy (World Health Organization (WHO), 2010; Baciewicz et al., 2013; Zumla et al., 2013). Isoniazid is known as an inhibitor of many human CYPs *in vitro* (Wen et al., 2002; Polasek et al., 2004) and clinically (Ochs et al., 1981, 1983).

Both the inductive effects of rifampicin and inhibitory effects of isoniazid on human CYPs have been extensively reported *in vitro* and *in vivo*. However, the data of their effects on human UGTs is limited. Furthermore, the information on other anti-TB drugs is also limited. In this work, inhibitory effects of isoniazid and rifampicin on human hepatic UGTs were studied; and inhibitory properties of the selected anti-TB drugs, including pyrazinamide, ethambutol, rifabutin, and bedaquiline were also studied *in vitro* with human hepatic CYP and UGT enzymes. Acetaminophen is widely used as an analgesic and antipyretic agent. Since APAP glucuronidation is the pathway responsible for converting two-thirds of a dose of APAP into non-toxic glucuronide conjugates, we also evaluated the inhibitory effect of the anti-TB drugs on acetaminophen glucuronidation.

Materials and Methods

Chemicals and solvents were purchased from Sigma-Aldrich Corp (St. Louis, MO) and Fisher Scientific (Pittsburg, PA). Isoniazid [Synonym: 4-Pyridinecarboxylic acid

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ABBREVIATIONS: APAP, acetaminophen; CYP, cytochrome P450; DDI, drug-drug interactions; DMSO, dimethyl sulfoxide; FDA, U.S. Food and Drug Administration; HLM, human liver microsome; K_i, the inhibition constant for the inhibitor; MeOH, methanol; MgCl₂, magnesium chloride; NADP, nicotinamide adenine dinucleotide phosphate; TB, tuberculosis; UDPGA, uridine 5'-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase; WHO, World Health Organization.

hydrazide], rifampin [Synonym: rifampicin, or 3-(4-Methylpiperazinyliminomethyl)-rifamycin SV], pyrazinamide, ethambutol hydrochloride [Synonym: 2,2'-(1,2-Ethanediyldiimino)bis-1-butanol dihydrochloride], and rifabutin [Synonym: Mycobutin] were purchased from Sigma-Aldrich Corp. Bedaquiline [a mixture of diastereomers, Synonym: 6-Bromo- α -[2-(dimethylamino)ethyl]-2-methoxy- α -1-naphthalenyl- β -phenyl-3-quinolineethanol] was purchased from Toronto Research Chemicals Inc. (North York, Canada). Water was purified with a Milli-Q system (Millipore Corporation, Milford, MA).

Liver samples from individual human donors with no known liver disease were provided by the International Institute for the Advancement of Medicine (Exton, PA), the Liver Tissue Procurement and Distribution System, University of Minnesota (Minneapolis, MN), or the National Disease Research Interchange (Philadelphia, PA). HLMs were prepared as previously described (von Moltke et al., 1993a; Greenblatt et al., 2011). Fifty-three individual liver microsomal preparations were combined to make a batch of pooled HLMs, by mixing an equal amount of protein from each HLM.

Inhibition Studies on CYP-Mediated Oxidation Using HLMs. Previously published incubation procedures using HLMs (Sonnichsen et al., 1995; Hesse et al., 2000; Giancarlo et al., 2001; von Moltke et al., 2001; Greenblatt et al., 2011) were used with modifications. Briefly, appropriate substrates and positive controls (Table 1) were added to incubation tubes. The anti-TB drugs were individually added in a series of concentrations to separate incubation tubes. Isoniazid, rifampicin, pyrazinamide, and ethambutol were at concentrations of 0, 10, 60, 100, 200, 400, 600 and 1000 μ M; rifabutin was at concentrations of 0, 10, 60, 100, 200, 400, and 600 μ M, except for CYP2C9 and 2D6 with an extra concentration of 1000 μ M; and bedaquiline was at concentrations of 0, 0.78, 1.56, 3.13, 6.25, 12.5, 20 and 25 μ M. The solvent (methanol) was evaporated to dryness at 40°C under mild vacuum conditions. Due to their poor solubility in methanol, propofol (the UGT1A9 substrate) and bedaquiline were prepared in DMSO and added directly to incubation tubes (1% DMSO v/v). Methanol at 1% (v/v) in the final incubation mixture was added to reconstitute the anti-TB compounds (except for bedaquiline) after dryness. The incubation mixtures for CYP-mediated oxidation contained 50 mM phosphate buffer (pH 7.5), 5 mM MgCl₂, 0.5 mM NADP, isocitrate and an isocitric dehydrogenase regenerating system, and appropriate amounts of the pooled HLMs. The anti-TB drugs were preincubated with HLMs (without the index substrates) for 20 min at 37°C, and then followed by another timed incubation with the substrates (250 μ l). All incubations were performed in duplicate. Initial tests for detecting IC₅₀ shifts were carried out by comparing incubations with 20 min preincubation to incubations without preincubation. One hundred microliters of acetonitrile (or acidified acetonitrile adjusted with 85% H₃PO₄ for CYP2B6 and 2C9) with internal standards were used to stop the reactions. After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV or HPLC-fluorescence analysis.

Inhibition Studies on Glucuronidation Using HLMs. Previously described incubation procedures were used with modifications (von Moltke et al., 1993b; Court, 2005, 2010). The incubation mixtures for the glucuronidation studies were prepared with 50 mM phosphate buffer (pH 7.5), 5 mM MgCl₂, alamethicin

(50 μ g/mg protein), and appropriate amounts of the pooled HLMs. The mixtures were kept on ice for 5 min before use. UDPGA was freshly prepared separately in the phosphate buffer. The reactions were initiated by addition of the UDPGA solution (a final concentration of 10 mM) in the incubation mixtures (100 μ l). All incubations were performed in duplicate. The incubations were conducted without preincubation except for those with β -estradiol (UGT1A1), trifluoperazine (UGT1A4), and APAP, for which the incubations with 20 min preincubation were also conducted. The reactions were stopped by adding 40 μ l of acetonitrile (or acidified acetonitrile adjusted with 85% H₃PO₄ for UGT2B7 and APAP glucuronidation) with internal standards to the incubation mixtures. After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV analysis.

K_i Value for Reversible Enzymatic Inhibition. Inhibition of UGT1A4 by rifabutin was observed with an IC₅₀ value of 11 μ M, which is low enough to trigger a DDI concern. As there was no IC₅₀ shift with and without preincubation, the experimental design for reversible enzymatic inhibition (Greenblatt et al., 2011) was applied to determine the K_i value for rifabutin versus human UGT1A4 using pooled HLMs. Varying concentrations of the index substrate (trifluoperazine) at 0, 2, 5, 10, 20, 34.2, 72.4, 144.9 and 336.6 μ M were incubated at 37°C with pooled HLMs in presence of varying concentrations of the inhibitor (rifabutin), at 0, 1.25, 5, 10, 30, and 60 μ M respectively. Probenecid at 2.4 mM was used as the positive inhibitory control. After 30 min incubation, the reactions were stopped with 40 μ l of acetonitrile (in the incubation mixtures of 100 μ l) with the internal standard (phenacetin). After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV analysis.

Analytical Methods

Previously described methods, with modifications were used for analysis of the in vitro samples in this study (von Moltke et al., 2001; Court, 2005). The HPLC conditions and detection methods are summarized (Supplemental Table 1). APAP glucuronide generated from the in vitro incubations was analyzed using the previously described method, with modifications (Zhao et al., 2015). Briefly, the HPLC analysis was carried out using a Hydro-RP column (4 μ m, 250 $\times$ 4.6 mm, Synergi Hydro-RP; Phenomenex, Torrance, CA), with a flow rate of 1.2 ml/min. The injection volume was 30 μ l, and the UV detection wavelength was 254 nm. A multistep gradient was started at 96.5% mobile phase A (20 mM potassium phosphate buffer, pH 2.2) and 3.5% mobile phase B (methanol) for 5 min, increased to 16% B during the next 5 min, and reached 20% B at 15 min, then to 40% B at 30 min, followed by a 9 min isocratic run at 100% mobile phase C (50% H₂O, 50% methanol), followed by another 10 min isocratic run at 3.5% B. The integration and quantitation were done with the software Chemstation (Agilent, Santa Clara, CA).

TABLE 1

In vitro systems using HLMs for evaluating inhibitory activities of the selected anti-TB drugs on human CYPs and UGTs

Enzyme Isoform	Substrate (Conc.)	Internal standard	Metabolite assayed	Inhibitor	Protein Conc. (μ g/ml)	Incubation time (min)
CYP1A2	Phenacetin (100 μ M)	2-acetaminophenol	Acetaminophen	α -Naphthoflavone	250	20
CYP3A	Triazolam (250 μ M)	Phenacetin	α -Hydroxytriazolam	Ketoconazole	250	20
CYP2B6	Bupropion (80 μ M)	2-acetaminophenol	Hydroxybupropion	Clopidogrel	333	20
CYP2C8	Taxol (25 μ M)	Phenacetin	6-Hydroxytaxol	Quercetin	300	20
CYP2C9	Flurbiprofen (5 μ M)	Naproxen	4'-Hydroxyflurbiprofen	Sulfaphenazole	250	20
CYP2C19	S-mephenytoin (25 μ M)	Phenacetin	4'-Hydroxymephenytoin	Ticlopidine	500	40
CYP2D6	Dextromethorphan (25 μ M)	Pronethalol	Dextropropan	Quinidine	250	20
CYP2E1	Chlorzoxazone (50 μ M)	Phenacetin	6-Hydroxychlorzoxazone	Diethyldithiocarbamate	250	20
UGT1A1	β -Estradiol (100 μ M)	Phenacetin	Estradiol-3-glucuronide	Probenecid	500	30
UGT1A4	Trifluoperazine (200 μ M)	Phenacetin	Trifluoperazine-glucuronide	Probenecid	250	30
UGT1A6	Serotonin (4 mM)	Phenacetin	Serotonin-glucuronide	Probenecid	50	30
UGT1A9	Propofol (100 μ M)	Phenacetin	Propofol-glucuronide	Niflumic acid	250	30
UGT2B7	3'-azidothymidine (AZT) (500 μ M)	3-acetaminophenol	AZT-glucuronide	Probenecid	500	120
UGT2B15	Oxazepam (100 μ M)	Phenacetin	S-oxazepam-glucuronide	Niflumic acid	500	120
APAP Glucuronidation	Acetaminophen (0.6 mM)	3-acetaminophenol	APAP-glucuronide	Probenecid	500	120

Fig. 1. In vitro inhibitory effects of rifabutin on human hepatic CYPs. (A) CYP1A2, (B) CYP3A, (C) CYP2B6, (D) CYP2C8, (E) CYP2C9, (F) CYP2C19, (G) CYP2D6 and (H) CYP2E1. The incubations were with preincubation (closed circle) and without preincubation (open circle). Data points represent the means ± S.E. of each concentration of rifabutin that was tested in duplicate. IC₅₀ values were determined by non-linear regression and summarized in Table 2.

Data Analysis

IC₅₀ Values. IC₅₀ values were determined using nonlinear regression as described previously (von Moltke et al., 2001; Greenblatt et al., 2011). Sigmaplot 11.0 was applied for the nonlinear regression procedure. Briefly, the relationships between the formation of the metabolites of the substrates and the inhibitory concentrations of the tested anti-TB drugs were analyzed by nonlinear regression fitting using eq. 1. The IC₅₀ values were then generated from the IC values using eq. 2 to take into consideration the possibility of incomplete inhibition.

$$R = 100 \left(1 - \frac{E_{\max} [I]^b}{[I]^b + IC^b} \right) \quad (1)$$

$$IC_{50} = \frac{IC}{(2E_{\max} - 1)^{1/b}} \quad (2)$$

R is the formation rate of the metabolite of interest, expressed as a percentage fraction of the control reaction velocity with no inhibitor; $E_{\max}$, the maximum degree of inhibition; $[I]$, the concentration of the anti-TB drugs; b , an exponent; IC , the inhibitor concentration producing an R value of 50% of $(100 - E_{\max})$, as determined from the nonlinear regression procedure; IC_{50} , the concentration of the tested anti-TB drugs producing 50% inhibition compared with the inhibitor-free control value, as calculated from the IC value using eq. 2.

K_i Value for Reversible Enzymatic Inhibition. The K_i value for rifabutin on human UGT1A4 was determined by nonlinear regression using the reversible inhibition model of full competitive inhibition (Greenblatt et al., 2011) on Sigmaplot 13.0. Significant substrate inhibition of trifluoperazine was observed at the concentration of 336.6 μM in our study, as reported previously (Uchaipichat et al., 2006). Thus, the reversible model of full competitive inhibition was fitted using concentrations of trifluoperazine up to 144.9 μM.

Results

IC₅₀ Values for Rifabutin on Human Hepatic CYPs. Rifabutin inhibited human CYP3A, 2B6, 2D6, 1A2, 2C8 and 2C9 to varying degrees in vitro using the pooled HLMs (Fig. 1; Table 2). At the

highest tested concentration (600 μM), no inhibition of human CYP2E1 or 2C19 was observed in vitro with rifabutin (Fig. 1).

IC₅₀ Values for Rifabutin on Human Hepatic UGTs, and K_i Value for Rifabutin on UGT1A4. Rifabutin inhibited UGT1A1, 1A4 and 2B15 to varying degrees (Figs. 2 and 3; Table 2), and partially inhibited human UGT1A9 and 2B7 (Fig. 2; Table 2) at a high concentration of 600 μM. The IC₅₀ values for rifabutin on human hepatic UGT1A4 were 10.8 and 11.3 μM respectively, for the incubations with and without preincubation. The K_i value for rifabutin on UGT1A4 using trifluoperazine as the index substrate was 2 μM, with the pattern of inhibition consistent with reversible competitive inhibition (Fig. 4).

IC₅₀ Values for Rifampicin and Isoniazid on Human Hepatic UGTs. Rifampicin had inhibitory effects on UGT1A1, 1A4 and 2B15 with varying IC₅₀ values (Fig. 3); partial inhibition of human UGT1A6 was observed at the highest tested concentration (1000 μM) (Fig. 2). No inhibitory effects of isoniazid were observed up to the highest tested concentration (1000 μM) (Fig. 2).

Inhibitory Effects of Pyrazinamide, Ethambutol and Bedaquiline on Human Hepatic CYPs and UGTs. Up to the highest tested concentration (1000 μM), no significant inhibitory effects of pyrazinamide or ethambutol were observed on the eight screened CYPs (Table 2) or six UGTs (Fig. 2). At the highest tested concentration (25 μM), bedaquiline partially inhibited human hepatic CYP3A, 2B6, 2C8, 2C19 and 2D6 at varied levels, but inhibition did not exceed 50% of the control metabolite formation rate (Fig. 2).

Inhibitory Effects of Anti-TB Drugs on APAP Glucuronidation. The IC₅₀ values for inhibition of APAP glucuronidation by rifabutin, with or without 20 min preincubation, were 237 and 422 μM respectively, and 860 and 545 μM respectively for rifampicin. Isoniazid, pyrazinamide, ethambutol, and bedaquiline produced minimal inhibition of APAP glucuronidation (Fig. 5; Table 2). The positive control (probenecid at 0.5 mM) produced approximately 50% inhibition of APAP glucuronidation.

Discussion

Rifabutin inhibited human CYP3A, 2B6, 2C8, 2D6, 1A2, 2C9, UGT1A1, 2B15, UGT1A9 and 2B7 in HLMs, with varying inhibitory potency. However, most of those inhibitory effects observed in vitro are

TABLE 2
IC₅₀ values of the selected anti-TB drugs on common human hepatic CYPs and UGTs

	Bedaquiline		Rifabutin		Pyrazinamide		Ethambutol		Rifampicin		Isoniazid	
	W ^a μM	W/O ^b μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM
CYP1A2	NC ^c	NC	NC	NC	NC	NC	NC	NC	—	—	—	—
CYP3A	NC	NC	27.9	31.5	NC	NC	NC	NC	—	—	—	—
CYP2B6	NC	NC	120.8	165.3	NC	NC	NC	NC	—	—	—	—
CYP2C8	NC	NC	64.0	62.6	NC ^d	NC	NC ^e	NC	—	—	—	—
CYP2C9	NC	NC	75	150.9	NC	NC	NC	NC	—	—	—	—
CYP2C19	NC	NC	NC	NC	NC	NC	NC	NC	—	—	—	—
CYP2D6	NC	NC	147.7	166.5	NC	NC	NC	NC	—	—	—	—
CYP2E1	NC	NC	NC	NC	NC	NC	NC	NC	—	—	—	—
UGT1A1	NC	NC	35	44	NC	NC	NC	NC	70	63	NC	NC
UGT1A4	^f	NC	10.8	11.3	—	NC	—	NC	—	230	—	NC
UGT1A6	—	NC	—	NC	—	NC	—	NC	—	^g	—	NC
UGT1A9	—	NC	—	NC	—	NC	—	NC	—	NC	—	NC
UGT2B7	—	NC	—	^h	—	NC	—	NC	—	NC	—	NC
UGT2B15	—	NC	—	81.3	—	NC	—	NC	—	357	—	NC
APAP-Glucuronidation	NC	NC	237.2	422.2	NC	NC	NC	NC	860	545	NC	NC

^aIncubations with preincubation.

^bIncubations without preincubation.

^cNot calculated (No IC₅₀ values were obtained due to less than 50% inhibition at the highest tested concentrations: 1000 μM for rifampicin, pyrazinamide, ethambutol, and isoniazid; 600 μM for rifabutin, and 25 μM for bedaquiline).

^d41% inhibition at 1000 μM.

^e37% inhibition at 1000 μM.

^fnot tested.

^g58% inhibition at 1000 μM.

^h57% inhibition at 600 μM.

Fig. 2. In vitro inhibitory effects of the selected anti-TB drugs on human hepatic UGTs. (A) UGT1A1, (B) UGT1A4, (C) UGT1A6, (D) UGT1A9, (E) UGT2B7, and (F) UGT2B15. Two concentrations of each compound were tested. z: Control without inhibition (black: 0 μ M); a: Pyrazinamide (black: 100 μ M, gray: 1000 μ M); b: Ethambutol (black: 100 μ M, gray: 1000 μ M); c: Rifabutin (black: 100 μ M, gray: 600 μ M); d*: Bedaquiline in (A) and (B), (black: 26 μ M, gray: 52 μ M); d: Bedaquiline (black: 12.5 μ M, gray: 25 μ M); e: Rifampicin (black: 100 μ M, gray: 1000 μ M); f: Isoniazid (black: 100 μ M, gray: 1000 μ M)

Fig. 3. In vitro inhibitory effects of rifabutin and rifampicin on human hepatic UGTs. (A) Rifabutin with UGT1A1; the concentrations of rifabutin are 0, 10, 60, 100, 200, 400 and 600 μM . (B) Rifabutin with UGT1A4; the concentrations of rifabutin are 0, 5, 10, 60, 100, 200 and 400 μM . (C) Rifabutin with UGT2B15; the concentrations of rifabutin are 0, 5, 10, 60, 100, 200 and 400 μM . (D) Rifampicin with UGT1A1; the concentrations of rifampicin are 0, 10, 60, 100, 200, 400, 600 and 1000 μM . (E) Rifampicin with UGT1A4; the concentrations of rifampicin are 0, 5, 10, 60, 100, 200, 400, 600 and 1000 μM . (F) Rifampicin with UGT2B15; the concentrations of rifampicin are 0, 10, 60, 100, 200, 400, 600 and 1000 μM . The incubations were with preincubation (closed circle) and without preincubation (open circle). Data points represent the means $\pm$ S.E. of each drug concentration that was tested in duplicate. IC_{50} values were determined by non-linear regression and summarized in Table 2.

Fig. 4. Rates of formation of trifluoperazine glucuronide in presence of inhibitory rifabutin (Rif). The K_i value for rifabutin on human hepatic UGT1A4 using trifluoperazine as the index substrate is 2 μM ($K_m = 77.6 \mu\text{M}$, $V_{\text{max}} = 3.6$). Data points represent the means $\pm$ S.E. of duplicates.

not likely to be of clinical importance, since the IC_{50} values were much higher than the reported maximum clinical plasma concentration of rifabutin of approximately 1.1 μM (Skinner et al., 1989; Peloquin, 2002).

Rifabutin inhibition of UGT1A4 ($K_i = 2 \mu\text{M}$ in pooled HLM) is potentially clinically relevant. Based on FDA guidance (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), 2012), an approximate estimation of the anticipated clinical DDI was calculated using the ratio of $[I]/K_i$ where $[I]$ is the maximum in vivo plasma concentration of rifabutin (1.1 μM) (Skinner et al., 1989) and K_i was 2 μM in this estimation. The ratio of 0.55 indicates a possibility that rifabutin may increase the systemic exposure of some drugs which are metabolized mainly by human UGT1A4. On the other hand, it has been widely reported that rifabutin induces human CYPs and UGTs (Baciewicz et al., 2013). Thus the prediction of the overall drug-drug interaction of rifabutin needs to consider both its inhibitory and possible inductive properties.

Human UGT1A1 is the principal metabolizing enzyme for several anti-HIV drugs such as raltegravir (Kassahun et al., 2007) and dolutegravir (Castellino et al., 2013). The IC_{50} values for rifabutin and rifampicin on UGT1A1 were approximately 35 and 70 μM respectively (Fig. 3). However, the inhibitory effects of rifabutin and rifampicin are not of major clinical importance, as their overall effects show predominantly inductive properties. In clinical studies, rifampicin significantly decreased the systemic exposure of dolutegravir (Dooley et al., 2013). Co-administration of rifabutin, on the other hand, did not alter the pharmacokinetics of raltegravir (Brainard et al., 2011) or dolutegravir (Dooley et al., 2013).

The IC_{50} values for rifabutin on CYP1A2 demonstrated a leftward shift (smaller IC_{50}) between the incubations without and with preincubation, consistent with time-dependent inhibition (Fig. 1). We did not determine the rate constant for inactivation in this study. Nevertheless, to our knowledge, no clinically meaningful DDIs due to inhibition of CYP1A2 by rifabutin have been reported.

The lack of significant inhibition of UGTs by isoniazid is reassuring for the use of isoniazid for latent TB treatment in HIV-infected patient receiving integrase strand transfer inhibitors (INSTIs)-based antiretroviral therapy. Isoniazid for 6 or 9 months is one of the preferred regimens for the treatment of latent TB (Getahun et al., 2015). The INSTIs such as

Fig. 5. (A) In vitro inhibitory effects of the tested anti-TB drugs on APAP glucuronidation using HLMs. Two concentrations of each compound were tested. z: Control without inhibitor (black: 0 μM); p: Probenecid as the positive control (0.5 mM); a: Pyrazinamide (black: 100 μM , gray: 1000 μM); b: Ethambutol (black: 100 μM , gray: 1000 μM); c*: Rifabutin (black: 80 μM , gray: 600 μM); d*: Bedaquiline (black: 26 μM , gray: 52 μM); e*: Rifampicin (black: 80 μM , gray: 1000 μM); f: Isoniazid (black: 100 μM , gray: 1000 μM). (B) In vitro inhibitory effects of rifabutin on APAP glucuronidation. The concentrations of rifabutin are 0, 8, 47.7, 79.6, 159.1, 318.3, and 477.4 μM . (C) In vitro inhibitory effects of rifampicin on APAP glucuronidation. The concentrations of rifampicin are 0, 8, 47.7, 79.6, 159.1, 318.3, 477.4 and 795.7 μM in incubations with preincubation (closed circle) and 0, 8, 47.7, 79.6, 159.1, 318.3, and 1000 μM in incubations without preincubation (open circle). Data points represent the means $\pm$ S.E. of each drug concentration that was tested in duplicate. IC_{50} values were determined by non-linear regression and summarized in Table 2.

dolutegravir, raltegravir or elvitegravir that are primarily metabolized by UGTs are essential components of preferred first-line antiretroviral therapy for HIV infection (Günthard et al., 2016). The findings in this in vitro study suggest that no dose adjustment of the INSTIs is necessary when co-administered with isoniazid, although in vivo studies may be needed to confirm this.

Incomplete inhibition was observed for a number of the CYP and UGT isoforms in this study. This might be explained by the participation of multiple isoforms in a given biotransformation pathway, particularly if the substrate is not specific for the target enzyme isoform of interest. The incomplete inhibition in Fig. 5, B and C could be explained by this. However, the explanation for incomplete inhibition in Fig. 3E is not clear, since the substrate trifluoperazine is reported to be highly specific for UGT1A4 (Uchaipichat et al., 2006). In addition, the incomplete inhibition was only observed with rifampicin but not with rifabutin. In any case, the calculated IC_{50} values correctly represent the inhibitor concentration that reduces the metabolite formation to 50% of the inhibitor-free control value (fixed at 100%). In the scenario of incomplete inhibition (E_{max} less than 1.0 in eq. 1), the true IC_{50} is calculated from IC using eq. 2.

Since high concentrations of the anti-TB drugs were used in the incubations, 1% methanol was introduced to improve solubility. Bedaquiline was prepared in DMSO for better solubility and added directly to the incubation mixtures. Because methanol and DMSO may themselves inhibit metabolic activities of CYPs and UGTs, inhibitor-free controls were included using the same concentrations of these solvents to control for any solvent effects that might occur.

Acetaminophen is a common over-the-counter analgesic and antipyretic. The CYP mediated oxidation pathway produces the toxic intermediate *N*-acetyl-*p*-benzoquinone imine (Miner and Kissinger, 1979), known to be responsible for acetaminophen hepatotoxicity. Parallel glucuronidation and sulfation of APAP are the major metabolizing pathways for generation of non-toxic metabolite conjugates. Several UGTs, including UGT1A1, 1A6, 1A9 and 2B15 are involved in APAP glucuronidation. (Court and Greenblatt, 2000; Court et al., 2001; Krishnaswamy et al., 2005; Mutlib et al., 2006). None of the selected anti-TB drugs significantly inhibited glucuronidation of APAP in vitro in this study (Fig. 5).

In conclusion, this study provides data on the inhibitory effects of anti-TB drugs on common CYPs and UGTs using HLMs in vitro. Rifabutin and rifampicin showed inhibitory properties to varying degrees. The findings for the other tested anti-TB drugs do not raise new concerns about clinical DDIs involving inhibition of hepatic CYPs and UGTs.

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Authorship Contributions

Participated in research design: Greenblatt, Kwara, Cao.

Conducted experiments: Cao.

Contributed new reagents or analytic tools: Greenblatt, Kwara.

Performed data analysis: Cao, Greenblatt, Kwara.

Wrote or contributed to the writing of the manuscript: Cao, Greenblatt, Kwara.

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