Full Paper

Inhibitory Effect of Antituberculosis Drugs on Human Cytochrome P450-Mediated Activities

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Abstract. The potential for drug-drug interactions mediated by the inhibition of cytochrome P-450 (CYP) were concerned during antituberculosis therapy. However, the information regarding human CYP inhibition by antituberculosis drugs is limited to isoniazid. In the current study, we examined the inhibitory effects of pyrazinamide and ethionamide, both of which are chemically related to isoniazid, on the CYP-mediated activities in human liver microsomes and compared them to that of isoniazid. No remarkable effects on any CYP activities were observed by pyrazinamide and ethionamide. In contrast, in addition to the reported inhibitory effect of isoniazid on CYP1A2, CYP2A6, CYP2C19, and CYP3A activities, our results newly showed its effect on CYP2C9 and CYP2E1 activities. Isoniazid showed potent direct inhibitory effect on *S*-warfarin 7-hydroxylation, while a preincubation step in the presence of NADPH was needed to inhibit chlorzoxazone 6-hydroxylation. Furthermore, irreversible inhibition of CYP2C19 activity by isoniazid was also observed in the dilution study. These results suggested that pyrazinamide and ethionamide did not seem to cause drug interactions mediated by the inhibition of CYP. In contrast, isoniazid might contribute to the severe drug interactions by a different inhibitory mechanism depending on each of the CYP isozymes, in addition to the reported observations.

Keywords: cytochrome P-450, antituberculosis drug, inhibition, drug interaction, human liver microsome

Introduction

The incidence of tuberculosis shows a tendency to increase over the past years because of the epidemic of human immunodeficiency virus infection. As the treatment of this infectious disease, antituberculosis drugs are prescribed for long periods of time and drug combination usually must be used to retard the development of resistant strains. Such a way of tuberculosis chemotherapy may lead to the high possibility of drug-drug interactions, which result from pharmacodynamic and pharmacokinetic modification of the drug response by the existence of concomitant drugs. Pharmacodynamic interactions are usually predictable, while pharmacokinetic interactions may not be anticipated. Many of the pharmacokinetic interactions are caused by

the induction or inhibition of the drug-metabolizing enzyme cytochrome P450 (CYP).

Rifampicin (RF) is well known as an inducer of several CYP isozymes, especially as a potent inducer of hepatic and intestinal CYP3A (1–4). Induction of CYP results in increased elimination of co-administered drugs, whose metabolism is mediated by the same isozymes, and this is often related to the insufficient pharmacological effects. There have been some reports showing such unexpected interactions by RF with clinically important drugs including cyclosporine, nifedipine, oral contraceptives, phenytoin, and theophylline (4, 5).

In contrast, the inhibition of CYP is involved in the increases of the plasma concentration and toxicity of concomitant drugs, especially which have narrow therapeutic range. Clinical trials and case reports have documented that isoniazid (INH) inhibited the metabo-

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lism of co-administered drugs such as acetaminophen, carbamazepin, diazepam, phenytoin, theophylline, and warfarin (6–11). It is considered to be caused by the inhibition of CYP (11–14). In a recent publication, Desta et al. have reported that INH was a potent inhibitor of CYP2C19 (competitive) and CYP3A (noncompetitive) in human liver microsomes (HLM) (13). Wen et al. also showed the inhibition of CYP1A2, CYP2A6, CYP2C19, and CYP3A4 activities by INH, and they further suggested that these effects were increased by the preincubation of INH with HLM at 37°C for 15 min in the presence of NADPH (14).

Among antituberculosis drugs, pyrazinamide (PZA) and ethionamide (TH) are chemically related to INH. PZA has a pyrazine moiety, while TH has a pyridine moiety, like INH does, in their chemical structure. Based on their similar structure to INH, we hypothesized that these two molecules could inhibit CYP. In fact, there has been a case report suggesting the possible modulation of CYP by PZA (15). The report showed that the combination use of INH and PZA increased the adverse reaction induced by phenytoin, whose metabolism is mainly mediated by CYP2C9 (15–17). Considering the minor inhibitory effect of INH on CYP2C9 activity as described by Desta et al. (13), it was predicted that the observed inhibition might be caused by PZA. Furthermore, the inhibitory effect of PZA on CYP-mediated metabolic activities has been also reported in rats (18). Facino et al. have shown that PZA inhibited aniline hydroxylase, *p*-nitroanisole *O*-demethylase, and aminopyrine demethylase activities in rat liver microsomes and explained these effects by the PZA-cytochrome P-450 (Fe^{2+})-binding characteristics (18). PZA is essential for the initial treatment of tuberculosis chemotherapy as well as INH and RF. TH also plays an important role as a second-line drug, which is used in the treatment of tuberculosis due to resistance to the first line drugs. Although they are frequently used in antituberculosis therapy, no studies have investigated their effects on human CYP.

In order to safely and effectively use antituberculosis drugs, it is important to study their effects on CYP. Therefore, the present study was conducted to investigate the inhibitory effects of PZA and TH on ethoxyresorufin *O*-deethylation (EROD), *S*-warfarin 7-hydroxylation (*S*-WF 7-OH), *S*-mephenytoin 4'-hydroxylation (*S*-MP 4'-OH), bufuralol 1'-hydroxylation (BF 1'-OH), chlorzoxazone 6-hydroxylation (CZX 6-OH), and midazolam 1'-hydroxylation (MDZ 1'-OH) for the estimation of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A activities, respectively. The inhibition of these CYP activities by INH was also studied to compare the potency of the effect with that of PZA and TH.

Materials and Methods

Chemicals

TH, INH, isonicotinic acid (INA), resorufin, glucose-6-phosphate (G-6-P), and 7-(β -hydroxypropyl) theophylline were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pyrazinamide, hydrazine monohydrochloride (HD), and nitrazepam were purchased from Wako Pure Chemical (Osaka). Ethoxyresorufin, *S*-warfarin, 7-hydroxywarfarin, *S*-mephenytoin, 4'-hydroxymephenytoin, bufuralol, 1'-hydroxybufuralol, 6-hydroxychlorzoxazone, and 1'-hydroxymidazolam were from Daiichi Pure Chemicals Co., Ltd. (Tokyo); glucose 6-phosphate dehydrogenase (G-6-PDH) and NADPH were from Oriental Yeast Co., Ltd. (Tokyo). Midazolam and chlorzoxazone were kindly donated by Nippon Roche Co., Ltd. (Tokyo) and from Dr. T. Inaba (Toronto University, Toronto, Canada), respectively. All other chemicals used were of analytical grade.

Human liver microsomes

A pooled sample of HLM (pooled from 10 donors) was purchased from Human and Animal Bridge Discussion Group (Chiba). The procedures of the study have all been reviewed and approved by the Ethical Committee of HAB and the Ethical Committee of Showa University.

Direct inhibitory effects of antituberculosis drugs on CYP activities

To investigate the direct inhibitory effects of PZA and TH on CYP activities and to compare their effects to that of INH, PZA (100 and 1000 μM), TH (10 and 100 μM), and INH (50 and 500 μM) were incubated with HLM and NADPH in the presence of the probe substrates of each CYP. The concentrations of antituberculosis drugs used in this study were determined based on their clinical steady state plasma concentrations or plasma maximal concentrations. TH was dissolved in 10% methanol (final concentration of MeOH, 1%), while PZA and INH were in water.

EROD, *S*-WF 7-OH, *S*-MP 4'-OH, BF 1'-OH, CZX 6-OH, and MDZ 1'-OH activities were assayed as the marker oxidation for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, respectively.

EROD was assayed fluorometrically according to the methods of Williams et al. (19) and Shinjari et al. (20) with slight modifications. Two hundred micrograms of HLM, 40 μM ethoxyresorufin, 2 mM NADPH, 100 mM MgCl_2 , and antituberculosis drugs in 200 mM potassium phosphate buffer, pH 7.4 were incubated at 37°C for 15 min (final volume of 500 μl). The reaction was stopped by the addition of 2 ml of ice-cold methanol

and the supernatant was used for the determination of resorufin using a fluorescence spectrophotometer (excitation, 530 nm; emission, 585 nm) (F-2000; Hitachi, Tokyo).

S-WF 7-OH was determined as follows: 200 μ g of HLM, 500 μ M S-warfarin, NADPH generating system (1 mM NADPH, 8 mM G-6-P, 0.5 U G-6-P DH, 40 mM MgCl_2) and antituberculosis drugs in 50 mM potassium phosphate buffer, pH 7.4 were incubated at 37°C for 30 min (final volume of 250 μ l). The reaction was stopped by the addition of 10 μ l of HClO_4 and the supernatant was injected in a high-performance liquid chromatography (HPLC) system equipped with fluorescence detector (excitation, 315 nm; emission, 415 nm). 7-Hydroxywarfarin was detected by a CAPCELLPAK C18 SG120 column (4.6 mm $\times$ 15 cm; Shiseido, Tokyo) and 0.5% phosphoric acid : acetonitrile (62:38) as mobile phase for 1.2 ml/min.

S-MP 4'-OH was determined according to the method of Chiba et al. with some modifications (21). The reaction mixture including 200 μ g of HLM, 100 μ M S-mephenytoin, NADPH-generating system (1 mM NADPH, 8 mM G-6-P, 0.5 U G-6-P DH, 40 mM MgCl_2), and antituberculosis drugs were incubated at 37°C for 60 min in a final volume of 500 μ l of 100 mM potassium phosphate buffer, pH 7.4. After the reactions were quenched by adding 200 μ l of acetonitrile, 2 μ g/ml phenobarbital sodium was added as the internal standard. The metabolite was extracted by 3 ml of dichloromethane and the organic solvent layers were evaporated under a nitrogen stream at room temperature. The residue was dissolved in the mobile phase and 4'-hydroxymephenytoin was detected by HPLC using UV detector (204 nm), CAPCELLPAK C18 AG120 column (4.6 mm $\times$ 25 cm) (Shiseido), and 50 mM potassium phosphate buffer (pH 4.0) : acetonitrile (74:26) as mobile phase for 1 ml/min.

BF 1'-OH, CZX 6-OH, and MDZ 1'-OH were assayed using 15, 200, and 100 μ g of HLM and 10, 50, and 10 μ M of each substrate as the final concentration, respectively. Each metabolite was determined by HPLC as described previously with slight modification (22–24). Bufuralol, chlorzoxazone, and midazolam oxidized products were detected by using the CAPCELLPAK C18 SG120 column (4.6 $\times$ 250 mm, Shiseido). MDZ 1'-OH product was resolved using a linear gradient of 50% Buffer A (10 mM potassium phosphate buffer (pH 7.4) : MeOH : CH_3CN , 70:15:15) to 100% Buffer B (10 mM potassium phosphate buffer (pH 7.4) : MeOH : CH_3CN , 30:35:35) on the CAPCELLPAK C18 SG120 column.

All of the HPLC analyses were carried out using a system consisting of a PX-8020 system controller

(Tosoh, Tokyo), a CCPM-II pump (Tosoh), a DG-100 degasser (Eicom, Kyoto), a HPLC auto sampler 460 (Kontron Instruments, Zurich, Switzerland), a UV-8000 detector (Toso), a L-7485 fluorescence detector (Hitachi), and a CR-4A integrator (Shimadzu, Kyoto).

Control experiments were carried out in the same conditions but with addition of water or 10% ethanol (final concentration was 1%) instead of antituberculosis drugs. Data were expressed as percentage of the remaining activities of control experiments.

Preincubation of antituberculosis drugs with HLM

To determine the possible inhibitory effects of the metabolic intermediates of antituberculosis drugs on CYP activities, the preincubation experiments were performed as follows: PZA (1000 μ M), ETH (100 μ M), or INH (100 μ M) were preincubated with HLM and NADPH at 37°C for 15 min. After the preincubation step, the marker substrates of each CYP were added and the activities were assayed as described above.

Dilution experiments

In order to investigate the irreversible nature of CYP inhibition by INH, the incubation was carried out with or without dilution of the incubation mixture.

Primary incubation mixture including tenfold concentrated HLM and 100 μ M INH was preincubated with NADPH at 37°C for 15 min. Aliquots (100 μ l each) of the primary reaction mixture were transferred to 900 μ l of a secondary reaction mixture containing 40 μ M ethoxyresorufin, 100 μ M S-MP, 200 μ M CZX or 10 μ M MDZ, and fresh NADPH in phosphate buffer (pH 7.4) and incubated for each indicated time for their assays. The CYP activities were analyzed as described above.

Results

Direct inhibitory effects of antituberculosis drugs on CYP activities

We first examined the direct inhibitory effects of PZA, TH, and INH on each CYP-mediated metabolic activity. As shown in Table 1, no remarkable inhibition on CYP activities were observed by PZA and TH. In contrast, INH showed concentration-dependent inhibition of all CYP activities tested. Potent inhibition of S-WF 7-OH, and S-MP 4'-OH with the remaining activity of 60% of the control activity was observed by 50 μ M of INH, which is comparable to the clinical plasma concentration.

Table 1. Direct inhibitory effects of antituberculosis drugs on cytochrome P450 activities

		% of control activity					
		CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A
	(μ M)	EROD	S-WF 7-OH	S-MO 4'-OH	BF 1'-OH	CZX 6-OH	MDZ 1'-OH
PZA	100	99	100	97	95	97	99
	1000	93	96	97	97	104	97
TH	10	98	102	98	106	91	94
	100	96	86	90	103	90	90
INH	50	93	63	60	86	88	77
	500	57	14	11	49	38	9

Each antituberculosis drug was directly incubated with human liver microsomes and NADPH in the presence of each probe substrate of various CYP. All of the data are expressed as the percentage of each control activity (14.6, 18.1, 49.8, 35.6, 681, and 612 pmol·min⁻¹·(mg protein)⁻¹ for EROD, S-WF 7-OH, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH, respectively). Data are shown as the mean of duplicate determinations.

Preincubation effects of antituberculosis drugs on CYP activities

To determine if it is possible for PZA and TH to form the metabolic intermediates that inhibit CYP activities during incubation, each CYP activity was assayed with the inclusion of a 15-min preincubation step. The preincubation of PZA or TH with HLM in the presence of NADPH resulted in the weak inhibition of CZX 6-OH by PZA and S-WF 7-OH by TH, with the remaining activity of 77% and 84% of the control activity, respectively, while these activities did not change without the preincubation step (Fig. 1: A and B). However, these inhibitory effects of PZA and TH were independent of the presence of NADPH and preincubation time (data was not shown).

On the other hand, the increase of the inhibition potency of INH on EROD, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH were observed: 89% to 44%, 40% to 26%, 91% to 44%, and 51% to 42% of the control activity, respectively (Fig. 1C). Furthermore, these intensified inhibitory effects were not observed in the absence of NADPH (Fig. 2).

Irreversibility of CYP inhibition by INH

Dilution experiments were performed to clarify the observed inhibition of INH on EROD, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH without the competitive component. The inhibitory effect of INH on S-MP 4'-OH was not significantly reversed after tenfold dilution of the primary inactivated mixture, suggesting participation of an irreversible mechanism (Table 2). In contrast, the reversibility of CZX 6-OH and MDZ 1'-OH activities were observed after dilution under the same conditions. EROD reversed to some degree after the dilution step; however, the effect was insufficient compared to that on CZX 6-OH and MDZ 1'-OH.

Discussion

In the current study, we investigated the inhibitory effects of PZA and TH on CYP-mediated metabolic activities in HLM in comparison to the effect of INH. Our results showed that PZA and TH did not show any significant effect on tested activities in HLM, while INH inhibited these activities as reported previously and with some new findings.

The inhibition of CYP activities by PZA, however, has been reported in rats. Facino et al. showed that PZA (1 mM) inhibited aniline hydroxylase, *p*-nitro-anisole *O*-demethylase, and aminopyrine demethylase activities in rat liver microsomes, by 32%, 40%, and 30% of the control activity, respectively (18). These results suggested that PZA inhibited various isozymes of CYP including CYP2B, CYP2C, CYP2E1, and CYP3A2 in rats. Furthermore, they explained the observed results by the binding characteristics of PZA to CYP (Fe²⁺) which resulted in the formation of the catalytically inactive CYP-drug complex and thus elicits the inhibitory phenomena. At first, we examined the direct inhibitory effect of PZA by the incubation of this compound in the presence of marker substrates of each CYP. If competitive interaction would occur in the incubating tube, the decline of tested activity should be observed. Our results did not show any such direct inhibitory effect of PZA on all of the activities tested (Table 1). Secondly, mechanism-based inactivation is another mechanism of the inhibition. In the situation that PZA could bind to CYP without competition of CYP-marker substrates, PZA might inhibit CYP activities. In this case, more severe and persisting inhibition is predicted because of the formation of a stable PZA-CYP complex. Then we next investigated the preincubation effect, incubating PZA with HLM for 15 min before

Fig. 1. Preincubation effect of antituberculosis drugs on cytochrome P450 activities. Human liver microsomes were preincubated with PZA (1000 μ M) (A), TH (100 μ M) (B), or INH (100 μ M) (C) at 37°C for 15 min in the presence of NADPH, followed by assay of each CYP activities. Data are shown as the mean of duplicate determinations. Open and closed column show the percentage of each control activity without and with the preincubation step, respectively. Control activities were 10.7, 21.1, 33.7, 24.4, 761, and 642 pmol $\cdot$ min⁻¹ $\cdot$ (mg protein)⁻¹ for EROD, S-WF 7-OH, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH, respectively. EROD: ethoxyresorufin *O*-deethylation, S-WF 7-OH: *S*-warfarin 7-hydroxylation, S-MP 4'-OH: *S*-mephenytoin 4'-hydroxylation, BF 1'-OH: bufuralol 1'-hydroxylation, CZX 6-OH: chlorzoxazone 6-hydroxylation, MDZ 1'-OH: midazolam 1'-hydroxylation.

addition of marker substrates. However, the results did not show any remarkable inhibition of CYP activities, except CZX 6-OH mediated by CYP2E1 was inhibited

by 20% compared to the control activity (Fig. 1A). To confirm if PZA was a mechanism-based inhibitor of CYP2E1, we conducted further experiments. The inhibition of CZX 6-OH did not depend on preincubation time up to 30 min and the presence of NADPH, suggesting that it did not seem to be a mechanism-based inactivation (data was not shown). The discrepancy of the results from two studies might suggest the species difference between rats and humans. There have been only few reports concerning the clinical interactions mediated by the inhibition of CYP during antituberculosis therapy with PZA. We first speculated that phenytoin toxicity, resulting from the concomitant use of INH and PZA reported by Sandyk (15), might be caused by the inhibitory effects of PZA on CYP2C9. However, our results explained this interaction by the inhibitory effect of INH on CYP2C9, rather than that of PZA. The concentration of PZA used in the current *in vitro* study was considered to be similar or rather higher compared to the plasma or hepatic concentrations in humans. Then in the view of interactions mediated by the inhibition of CYP, PZA did not seem to cause interactions with concomitant drugs.

Chemicals with similar structures often show common characteristics as the substrates of specific CYP isozymes. Pyridine is considered to interact with CYP because of the compounds containing uncoupled pairs of electrons on the nitrogen atoms (25). Because TH has a pyridine moiety in its chemical structure, the same as INH, the effects of TH on CYP activities was also investigated to examine if the pyridine was the component for the potent inhibition against CYP. The results showed that no direct and mechanism-based inhibitory effects of TH on any CYP activities determined (Table 1, Fig. 1B). In fact, we could not find any clinical reports regarding drug interactions mediated by CYP inhibition during TH therapy. Further investigation is required concerning the chemical structural contribution for the inhibitory characteristics on CYP. It would be essential information for the development of new drugs and also for the prediction of drug-drug interactions.

In the current study, we also investigated the inhibitory effect of INH on CYPs as the contrast to that of PZA and TH. The inhibition of CYP by INH has been already documented by some authors, but we showed some new findings in our study. One of such results was the direct inhibitory effect of INH on CYP2C9 activity. As shown in Table 1, the potent inhibition of S-WF 7-OH by INH was observed even by the lowest concentrations tested (50 μ M) with its activity remaining at approximately 60% of the control activity, and this effect was equal to that on CYP2C19 activity. In con-

Fig. 2. NADPH requirement for the inhibitory effect of isoniazid on cytochrome P450 activities. Human liver microsomes were preincubated with 100 μ M isoniazid at 37°C for 15 min in the presence or absence of NADPH, following by assay of each CYP activity. Data are shown as the mean of duplicate determinations. Open, striped, and closed column show the percentage of each control activity after no preincubation of INH with NADPH, preincubation for 15 min with NADPH, and preincubation for 15 min without NADPH, respectively. Control activities were 10.6, 17.3, 35.7, 46.1, 753, and 687 $\text{pmol} \cdot \text{min}^{-1} \cdot (\text{mg protein})^{-1}$ for EROD, S-WF 7-OH, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH, respectively.

Table 2. Effect of dilution on the inhibition of cytochrome P450 activities by isoniazid

	% of control activity			
	CYP1A2	CYP2C19	CYP2E1	CYP3A
	EROD	S-MP 4'-OH	CZX 6-OH	MDZ 1'-OH
Without dilution after preincubation	50	25	51	45
10-fold dilution after preincubation	73	35	90	91

Human liver microsomes were preincubated with 100 μ M isoniazid at 37°C for 15 min in the presence of NADPH, following by tenfold dilution for assay of each CYP activities. All of the data are expressed as the percentage of each control activity (10.1, 23.3, 893, and 589 $\text{pmol} \cdot \text{min}^{-1} \cdot (\text{mg protein})^{-1}$ for EROD, S-MP 4'-OH, CZX 6-OH, and MDZ 1'-OH, respectively). Data are shown as the mean of duplicate determinations.

trast, previous studies showed only a minor effect of INH on CYP2C9 activity (13, 14). This difference might be attributed to the difference of the probe substrate used as CYP2C9-mediated metabolic activity; we assayed S-WF 7-OH, while the previous studies were performed by assaying tolbutamide 4-methylhydroxylation. Currently, there are some clinical reports suggesting the possibility of the inhibition of CYP2C9 by INH. For example, INH has been documented to increase phenytoin serum concentrations and toxicity (8, 11, 15). In addition, another case report showed the prolongation of prothrombin time by the result of the concomitant use of warfarin and high dose of INH (600 mg/day) (10). The metabolism of phenytoin and warfarin are mainly mediated by CYP2C9, although CYP2C19 also contributed to the oxidation of phenytoin (17). These clinical observations and our results suggested that INH

might cause the adverse reaction of co-administered drugs, whose metabolism is mediated by CYP2C9 in addition to that by CYP2C19.

Another effect of INH we newly showed in the current study was the inhibition of CYP2E1 activity after the preincubation step. Although the inhibition of CZX 6-OH by INH was minor without preincubation of INH (91% of control activity), the effect was intensified with preincubation for 15 min (44% of control activity) (Fig. 1C). Furthermore, this effect was dependent on the presence of NADPH (Fig. 2). These results indicated the metabolic inactivation of INH on CYP2E1. At present, only a few clinical drugs whose biotransformations are mediated by CYP2E1 are known but it includes some clinically important drugs such as acetaminophen, chlorzoxazone, and enflurane (26–28). It has been reported that INH markedly inhibited the metabolism

of chlorzoxazone to 6-hydroxychlorzoxazone and acetaminophen to *N*-acetyl-*p*-benzoquinone (NAPQI) in humans (29–31). Our results suggested that these observations were caused by the inhibition of CYP2E1 activity through the metabolic inactivation of the enzyme.

Furthermore, many clinically important CYP-mediated drug-drug interactions are not predictable by the classical approach that assumes reversible inhibition. Irreversible inhibition is an additional mechanism by which the catalytic activity of an enzyme may be reduced both in vitro and in vivo (32) and often resulted in severe drug-drug interactions and adverse events. For more correct prediction of drug-drug interaction, it is important to investigate if the inhibitory effects of the drugs are irreversible. However, this kind of approach has not been performed with INH. We next determined if the observed inhibition is reversible or irreversible. For the exception of competitive characteristics, after the incubation of INH with HLM in the presence of NADPH, we diluted the incubation mixture by tenfold and then assayed each CYP activity. The results of this study showed that the inhibitory effects of INH on CYP2C19 were not reversible, while that on CYP2E1 and CYP3A were reversible (Table 2). This suggested that more severe interaction might occur for the concomitant drugs, whose metabolism was mediated by CYP2C19. EROD activity did not reverse completely after the dilution step and the reversibility of inhibited CYP1A2 activity by INH was unclear. Further studies are required to confirm and investigate the characteristics of INH as a mechanism-based inactivator of CYP1A2 and CYP2C19.

In summary, this report demonstrated that PZA and TH did not seem to cause drug interactions with co-administered drugs by the inhibition of CYP, even though their chemical structures were similar to that of INH. To our knowledge, this is the first report that showed no inhibitory effects of PZA and TH on CYP activities in HLM. In contrast, INH might cause severe drug interactions with other drugs during anti-tuberculosis therapy by a different mechanism depending on each CYP isozyme. In addition to the inhibition of CYP1A2, CYP2A6, CYP2C19, and CYP3A activities, CYP2C9 and CYP2E1 might also participate in clinical drug interactions. Furthermore, because the inhibition of CYP2C19 activity by INH was irreversible, more severe and persisted inhibition was predicted. These results contribute essential information for the effective and safe use of medication for antituberculosis therapy.

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