185 The evaluation of induction and inhibition potency of various drugs on CYP3A4 and OATP1B1/1B3

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Introduction

It is well known that the drug-mediated induction of cytochrome P450s (CYPs) has important role in the clinical drug-drug interactions (DDI). In addition, it is reported that considering the induction of transporters is needed to predict the clinical DDI more accurately. The DDI guidance issued by the U.S. Food and Drug Administration (FDA) suggests to evaluate the induction potency of drugs on not only CYPs but also transporters[1], however, the evaluation is not performed frequently. Thus, we evaluated the induction and inhibition potency of CYP3A4 and organic anion transporting polypeptide (OATP) 1B1/1B3, that are induced by the same nuclear receptor, using various drugs related to DDI of CYP3A4 and OATP1B1/1B3.

Method

The following drugs were selected for this study.
<Test drugs>
Rifampicin, Cyclosporin A, Midazolam, Ketoconazol, Atorvastatin, Nifedipine, Rosuvastatin, Troleandomycin

<Induction study>
Cryopreserved human hepatocytes. (Lot No. HC5-30, Individual lot, Sekisui XenoTech) were prepared according to the manual of OptiTHAW Hepatocyte isolation kit (Sekisui XenoTech). Induction was performed according to the following procedure:
The entire volume of the solution in each well was removed, each drug solution or solvent control solution was immediately added. The plate was allowed to stand overnight in a CO_2 incubator. Each medium was refreshed at 24 hr of cultivation and removed at 48 hr. After induction treatment, RNA was extracted from the hepatocytes and mRNA was measured using a Real-Time PCR system (QuantStudio7, Thermo Fisher Scientific).

<Inhibition study on CYP3A4>
Incubation samples containing phosphate buffer (100 mM, pH 7.4), human liver microsomes (final concentration: 1 mg protein/mL, Sekisui XenoTech), NADPH generating system and each drug or solvent control, were incubated at 37° C for 30 min. The reaction was started by addition of the midazolam solution, and the incubation samples were incubated at 37°C for 10 min. The reaction was terminated by adding acetonitrile solution containing internal standard. The metabolite concentrations were determined by LC-MS/MS measurement. Remaining activity (% of control) of CYP3A4 was calculated to the following equation.

$$\%\ \text{of control} = \frac{\text{Peak area ratio in drugs group}}{\text{Peak area ratio in control group}} \times 100$$

<Inhibition study on OATP1B1/1B3>
The OATP1B1- or OATP1B3-expressing HEK293 cells and control cells were cultured in 75-cm^2 bottom flasks and subjected to passage every 3 to 5 days. The OATP1B1-, OATP1B3-expressing HEK293 cells and control cells were seeded in Collagen I-coated 24-well plates and incubated in a CO_2 incubator (37°C, CO_2: 5%) for 1 day, after that 0.5 mL of a medium containing 10 mmol/L butyrate was added, and the cells were further incubated for 1 day.
The entire medium in the plate seeded with OATP1B1- or OATP1B3- and control cells was removed and replaced with 1 mL of HBSS. HBSS was removed, replaced with 0.3 mL of the HBSS solution containing each drug or solvent control, and the plate was preincubated at 37°C for 30 min. After the preincubation, the buffer was replaced with 0.3 mL of probe substrate solution containing each drug or solvent control, and each mixture was incubated at 37°C for 2 min. After the incubation, each solution was removed, and the cells were washed with 1 mL of ice-cold PBS containing 0.2% BSA once and 1 mL of ice-cold PBS twice. The entire PBS solution was removed, and the cells were dissolved in 0.5 mL of 0.1 mol/L NaOH. After pipetting the cell solution, 0.3 mL of the cell lysates was collected into a vial and mixed with 5 mL of Emulsifier-Safe to measure the radioactivity using LSC.
Cleared volume and % of control were calculated to the following equation.

$$\text{Cleared volume} = \frac{\text{Uptake amount}}{\text{Protein amount x Initial concentration}}$$

$$\%\ \text{of control} = \frac{\text{D-C}}{\text{B-A}} \times 100$$

A: Cleared volume of control cells in the absence of drugs
B: Cleared volume of transporter-expressing cells in the absence of drugs
C: Cleared volume of control cells in the presence of drugs
D: Cleared volume of transporter-expressing cells in the presence of drugs

<Calculation of IC_{50} value>
We calculated IC_{50} values according to following equation if the drugs showed inhibition effects more than 50%.

$$\%\ \text{of control} = \frac{IC_{50}}{IC_{50} + I} \times 100$$

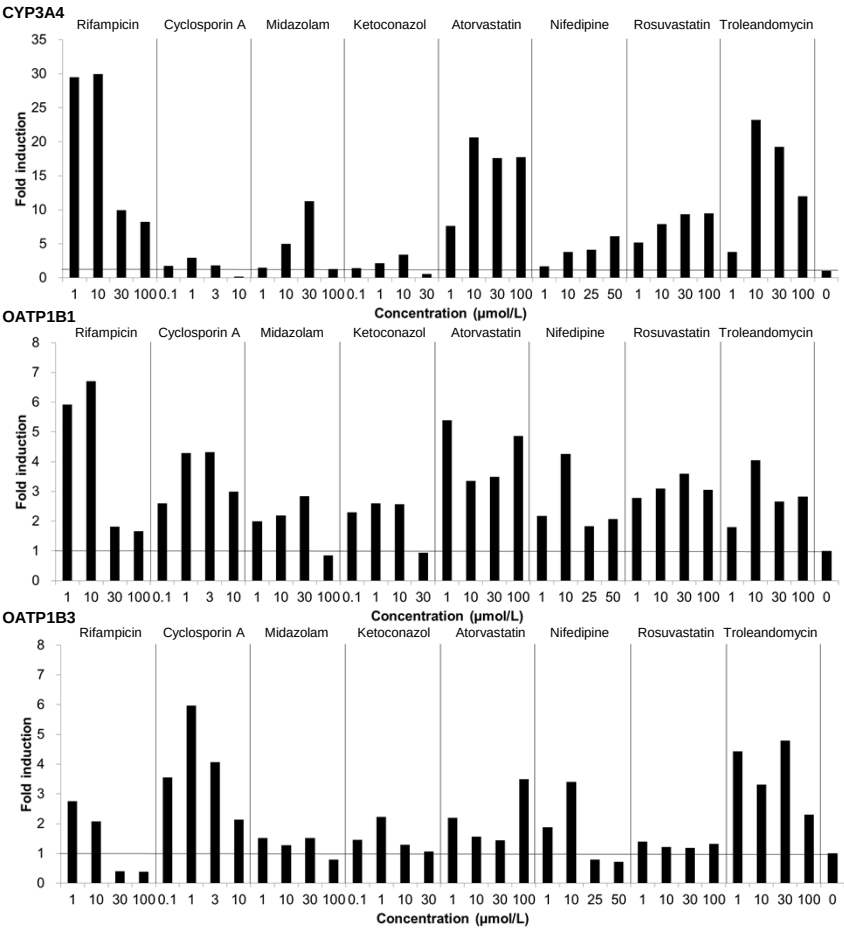
Discussion

We evaluated induction and inhibition potency of CYP3A4 and OATP1B1/1B3 using various drugs related to them. As for induction potency, we expected that the same nuclear receptor is involved in the drug-mediated induction of CYP3A4 and OATP1B1/1B3, and so the drugs which have induction potency of CYP3A4 also induce OATP1B1/1B3 on the same level as CYP3A4. Most of the drugs tested induced both CYP3A4 and OATP1B1/1B3. However the magnitude of induction of OATP1B1/1B3 tended to be lower than CYP3A4. This result suggests that they are regulated by the different nuclear receptors[2), 3)]. Moreover, we examined the relationship between induction and inhibition potency. Many drugs showed inhibition potency on CYP3A4 and OATP1B1/1B3 as well as induction potency. Asaumi et al., reported that considering induction effect of rifampicin on OATP1B1/1B3 leads to more accurate prediction of the clinical DDI caused by rifampicin[4)]. Therefore, the evaluation of induction potency of OATP1B1/1B3 will be important in the future, but considering the involvement of different nuclear receptors, CYP3A4 and OATP1B1/1B3 should be evaluated separately.

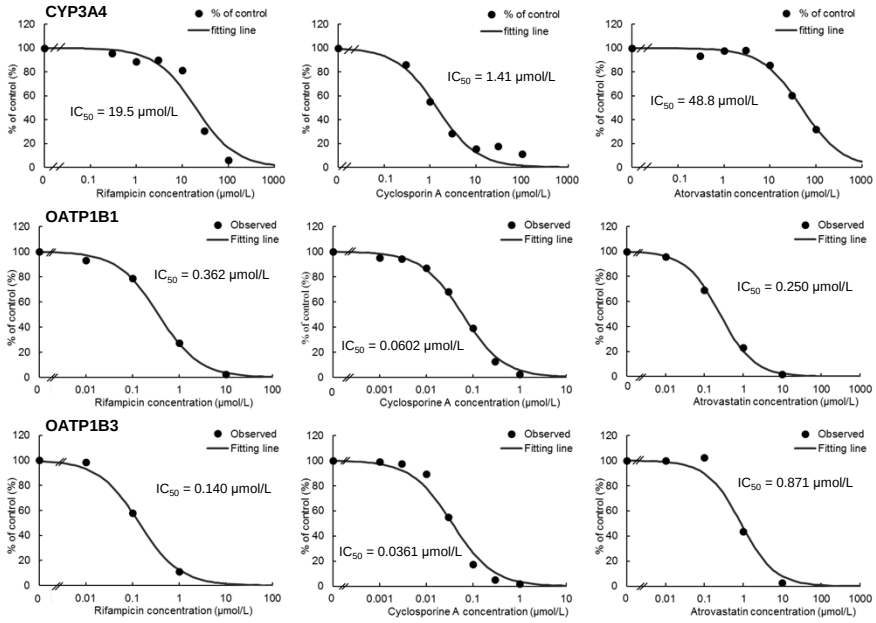
References

1) U.S. Food and Drug Administration. Guidance for Industry (DRAFT GUIDANCE): In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies Guidance for Industry (2017). 2) Hepatology. 2010;52(5):1797-807 3) Drug Metab Dispos. 2019;47(12):1433-1442 4) CPT Pharmacometrics Syst Pharmacol. 2019;8(11):1-13 5) Basic Clin Pharmacol Toxicol. 2005;97(4):249-256 6) Pharmacol Toxicol. 1999;85(4):157-161 7) Anaesthesia. 2005;60(8):747-753 8) Drug Metab Dispos. 2005;33(6):853-861 9) J Pharmacol Exp Ther. 2003;304(1):223-228 10) Eur J Pharmacol. 2008;584(1):57-65 11) Drug Metab Dispos. 2015;43(2):235-247 12) Eur J Pharmacol. 2008;584(1):57-65

Results



<Figure 1 Effect of drugs on CYP3A4, OATP1B1 and OATP1B3 mRNA expression in cryopreserved human hepatocytes>



<Figure 2 Inhibitory effect of drugs on CYP3A4 and OATP1B1/1B3>

<Table1 Relationship between induction and inhibition potency>
As induction potency, maximum fold induction and tested concentration were shown in the left and right column, respectively.
As inhibition potency, IC_{50} values with reference values in parentheses were shown.

Test drugs	Induction potency						Inhibition potency		
	Maximum fold induction with concentration (μmol/L)						IC_{50} value (μmol/L) and reference value (μmol/L)		
	CYP3A4		OATP1B1		OATP1B3		CYP3A4	OATP1B1	OATP1B3
Rifampicin	29.9	10	6.7	10	2.8	1	19.5 (18.5, Ki[5)])	0.362 (0.94~1.5[9, 10)])	0.140 (2.6[12)])
Cyclosporin A	2.9	1	4.3	3	6.0	1	1.41	0.0602 (0.0139[11)])	0.0361
Midazolam	11.3	30	2.8	30	1.5	1	N/A	22.0	65.1
Ketoconazol	3.4	10	2.6	1	2.2	1	0.03 (0.12[6)])	1.73	3.75
Atorvastatin	20.6	10	5.4	1	3.5	30	48.8 (12.4, Ki[7)])	0.250	0.871
Nifedipine	6.1	50	4.3	10	3.4	10	6.41	5.80	No inhibition
Rosuvastatin	9.5	100	3.6	30	1.4	1	42.0	3.58	6.22
Troleandomycin	23.2	10	4.1	10	4.8	30	1.14 (0.3, Ki[8)])	30.5	8.61