Is the Dilution Method for Measuring IC_{50} Shift Values a More Sensitive Approach for Evaluating Time-Dependent Inhibition?

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Poster#: P129

Abstract

In 2022, with the promulgation of ICH M12, the evaluation methods for TDI in drug interactions became elusive. The original regional guidelines such as the FDA did not specify the IC_{50} shift method, but the M12 guidelines pointed out that the dilution method should be used for the IC_{50} shift. This has sparked extensive discussions in the industry. Based on the internally validated IC_{50} shift system, we conducted a comparative study of the dilution and non-dilution methods.

Method

We used an internally validated system to ensure the same substrate concentration and reaction time during the incubation stage. The only difference was in the pre-incubation period: the dilution method used higher microsomal concentration (1.0 mg/mL) and compound concentration, then the solution was added to the incubation solution with a 10-fold dilution factor, while the non-dilution method used lower microsomal concentration (0.1 mg/mL) and compound concentration, and directly added to the incubation solution. We performed comparative tests with 3-5 commercially available compounds for each of the CYP isoforms recommended by the ICH M12 guideline.

Results

All the IC_{50} shift values generated in both the dilution and non-dilution methods were compared. It was found that the same results were obtained for most of the compounds tested regardless of the dilution and non-dilution methods. However, three exceptions were observed: Phenelzine on CYP2C8, and Ticlopidine on CYP3A (using midazolam and testosterone as substrates, respectively) (Table 1). By comparing the reported TDI levels of the compounds in the literature, we found that the dilution method produced one false negative (Phenelzine on CYP2C8) and two false positives (Ticlopidine on CYP3A (using midazolam and testosterone as substrates, respectively)), while the non-dilution method could accurately assess the time-dependent properties of the compounds. Since the inhibition experiment is sometimes affected by different substrates, here is an explanation of the substrates used for each isoform. The substrates are as follows: Phenacetin (CYP1A2), bupropion (CYP2B6), Amodiaquine (CYP2C8), Diclofenac (CYP2C9), S-mephenytoin (CYP2C19), Dextromethorphan (CYP2D6),Midazolam and Testosterone (CYP3A).

To further confirm the properties of the compounds, we conducted Kinact/KI experiments on the two exceptional compounds. The inactivation of CYP2C8 by phenelzine was characterized by a KI value of 199 μM and a kinact value of 0.0474min-1. However, no inactivation values of Ticlopidine could be generated. It turned out that the properties of the two compounds were correctly classified in the non-dilution method.

Table 1. Summary of the IC_{50} shift values obtained from the non-dilution and dilution methods

P450 Enzyme	Inhibitors	IC_{50} Shift Fold[a]	
		Non-Dilution	Dilution
CYP1A2	Fluvoxamine***	0.856 (-)	0.751 (-)
	Ticlopidine**	4.96 (+)	3.78 (+)
	Furafylline*	31.8 (+)	191 (+)
CYP2B6	Montelukast***	0.903 (-)	0.876 (-)
	Sertraline**	18.0 (+)	20.5 (+)
	Ticlopidine*	12.5 (+)	6.15 (+)
CYP2C8	Montelukast***	0.726 (-)	0.624 (-)
	Phenelzine**	14.1 (+)	1.21 (-)
	Gemfibrozil 1-O-β-Glucuronide*	17.7 (+)	59.5 (+)
CYP3A-M	**Ticlopidine*** **	**NA[b] (-)**	**>5.62 (+)**
	Ketoconazole***	0.613 (-)	0.460 (-)
	Paroxetine**	2.33 (+)	2.45 (+)
	Azamulin*	5.08 (+)	63.0 (+)
	Verapamil*	10.7 (+)	90.5 (+)
CYP3A-T	Ticlopidine***	1.17 (-)	3.11 (+)
	Ketoconazole***	0.572 (-)	0.517 (-)
	Paroxetine**	2.47 (+)	2.22 (+)
	Azamulin*	3.61 (+)	3.71 (+)
	Verapamil*	14.0 (+)	76.6 (+)

*: Potent TDI/FDA recommended; **: Moderate/Weak TDI; ***: No TDI.
Note: (-): no time-dependent inhibition, (+): time-dependent inhibition.
a: For dilution method, IC_{50} Shift Fold = IC_{50} (-NADPH) Final / IC_{50} (+NADPH) Final.
b: AUC shift = 13.5%; AUC shift < 15%, no TDI.

Conclusion

In summary, the dilution method for measuring IC_{50} shift values is not a more sensitive approach for evaluating time-dependent inhibition, we recommend using non-dilution method for its accuracy. Non-dilution method is included in the final version of ICH M12.

References

[1] FDA guidance for industry: In Vitro Drug Interaction Studies-Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions, January 2020

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