

Evaluation of direct and metabolism-dependent inhibition of Cytochrome P450 enzymes

Introduction

Cytochrome P450 (CYP) inhibition can occur due to two major types of inhibition, namely direct and time-dependent inhibition. In contrast to time-dependent inhibition, direct inhibition occurs without a significant delay (i.e. within seconds) and without requiring biotransformation. When the inhibitory potency of the drug candidate increases with incubation time, the inhibition type is called time-dependent inhibition. This type of inhibition may occur by different underlying mechanisms, e.g. slow-binding inhibition (reversible process in which initial inhibition becomes more potent over time), metabolism-dependent conversion of the drug candidate to a more potently acting direct inhibitor, or metabolism-dependent conversion of a drug candidate to a metabolite, that quasi-irreversibly coordinates with the heme-iron or covalently (irreversibly) binds to structures of the CYP enzyme, respectively.

Testing strategy

Figure 1 demonstrates a flow chart for initial CYP inhibition studies. If CYP inhibition has been observed during screening, the starting point should be an IC₅₀ experiment using human single recombinant CYPs or human liver microsomes with and without a 30 minute preincubation in presence and absence of NADPH.

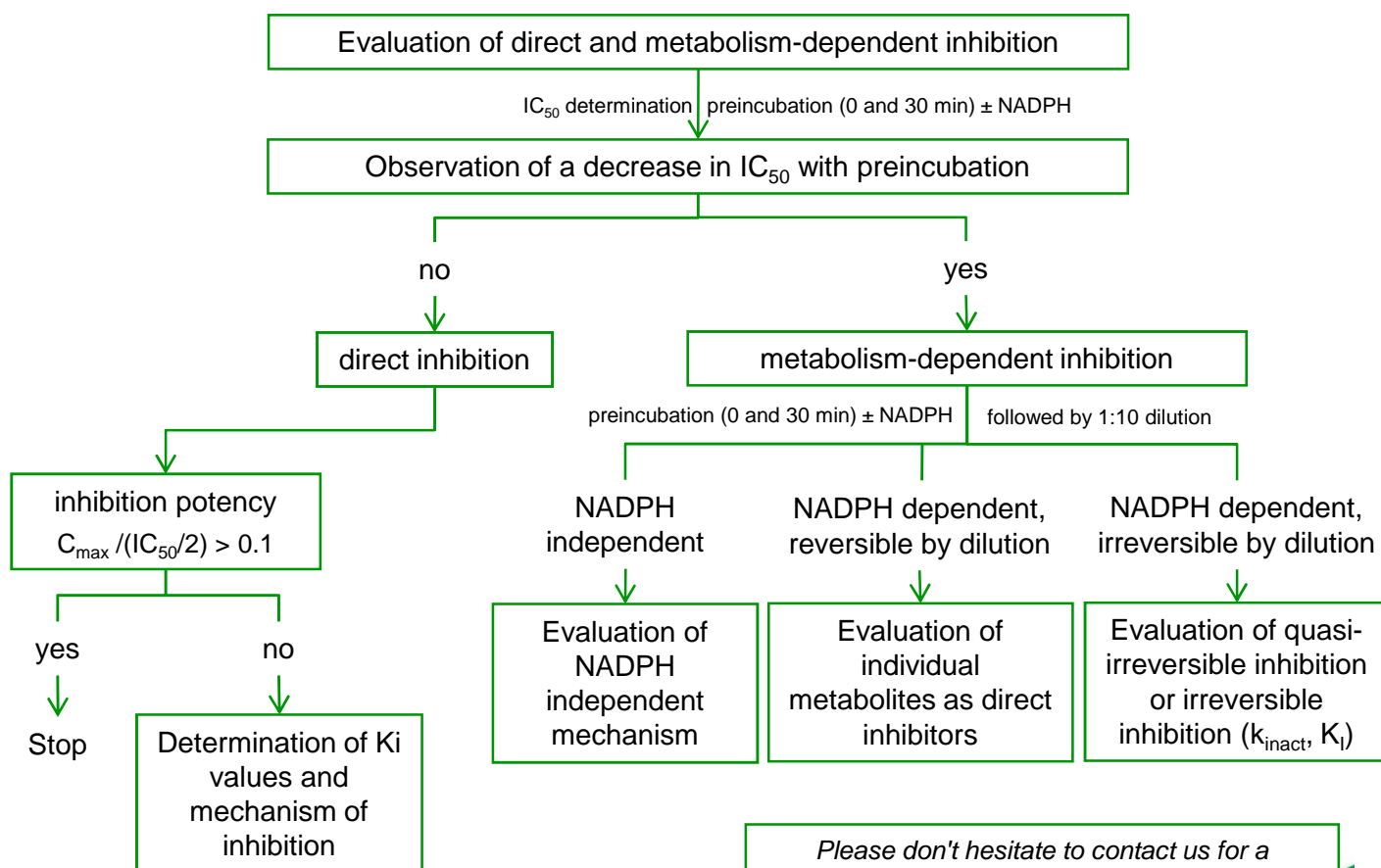


Figure 1: Decision tree for initial CYP inhibition studies [adapted from Ogilvie et al., in Drug-Drug Interactions, 2nd Edition, edited by A. David Rodrigues]

Date: 05/2009

Please don't hesitate to contact us for a customized quotation

Dr. Ursula Mueller-Vieira
Head of ADME & *in vitro* Pharmacology
Tel: +49 681 3946-7521
mueller@pharmacelsus.de
www.pharmacelsus.de

Example:

Troleandomycin as mechanism-dependent inhibitor of CYP3A4

Troleandomycin (TAO) was applied as a model substance for mechanism-dependent inhibition. This compound has been characterised as quasi-irreversible inhibitor of CYP3A4, since a metabolic step is required to convert troleandomycin into a nitroso-metabolite that coordinates with the heme iron of CYP3A4, associated with the formation of a metabolic-intermediate (MI) complex [Ogilvie et al. Drug-Drug Interactions, 2nd Edition]. The strategy shown in Figure 1 has been applied exemplarily for TAO.

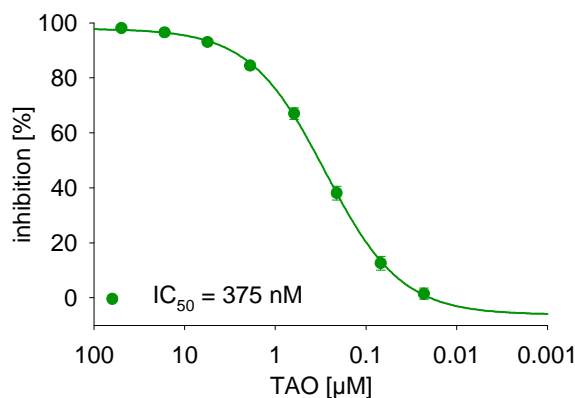


Figure 2: Direct inhibition of CYP3A4 by TAO determined using Supersomes™

Step 1: Direct inhibition

The IC₅₀ value of TAO was determined using human recombinant CYP3A4 (Supersomes™) and 7-benzoyloxytrifluoromethyl-coumarin (BFC) as substrate (Figure 2). The IC₅₀ was determined to be 375 ± 33 nM.

Step 2: NADPH dependence

Using the same test system, the dependency on NADPH was evaluated. Preincubations in the presence or absence of NADPH for 0 and 30 minutes were conducted. With preincubation in presence of NADPH, a decrease of the IC₅₀ to 151 ± 3 nM was observed, whereas in absence of NADPH, this effect was negligible (Figure 3).

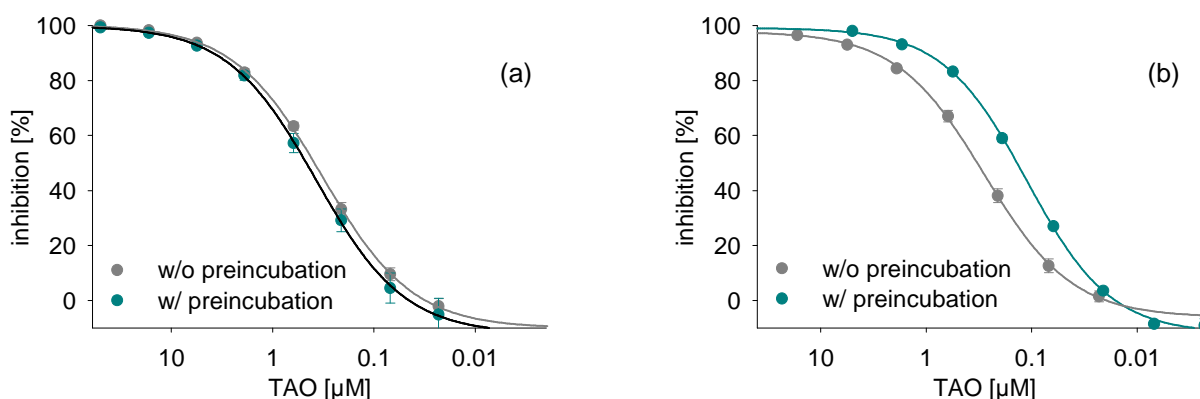


Figure 3: NADPH dependence: preincubation of TAO for 0 and 30 min absence (a) and presence (b) of NADPH.

Step 3: Irreversibility

In the next step, the same concentrations of TAO were preincubated for 0 and 30 minutes in presence of NADPH as well, but the samples were diluted 10-fold prior to measuring CYP3A4 activity. The increase of inhibition that is time- and NADPH-dependent and also resistant to dilution provides evidence that TAO acts as a (quasi-) irreversible, metabolism-dependent inhibitor of CYP3A4 (Figures 3 and 4).

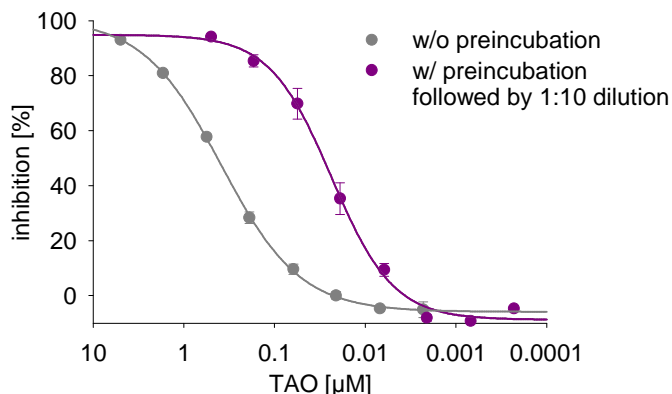


Figure 4: Determination of irreversibility by dilution