VALIDATION OF CYTOCHROME P450 TIME-DEPENDENT INHIBITION ASSAYS
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[Purpose] In the drug-discovery process, in vitro assessment of cytochrome P450 (CYP) inhibition is necessary to
predict drug-drug interactions (DDI) in clinical use. In addition, assessment of time-dependent inhibition (TDI)
is important for toxicity and DDI prediction in drug candidates. PhRMA recently recommended a standard dilution
method (determination of \( K_I \) and \( k_{\text{inact}} \)) and IC\(_{50}\) shift method for identification of time-dependent inhibitors. Thus, we
validated a test system for assessing potential time-dependent inhibitors by determining IC\(_{50}\) shift, \( K_I \), and \( k_{\text{inact}} \).

[Methods] Well-established time-dependent inhibitors were assayed by the IC\(_{50}\) shift method in human liver
microsomes. The enzyme / marker activities / inhibitor combinations are as follows: CYP1A2 / phenacetin
O-deethylation / furafylline, CYP2A6 / coumarin 7-hydroxylation / 8-methoxyspolaren, CYP2B6 /
propion hydroxylation / ticlopidine, CYP2C8 / amodiaquine N-deethylation / gemfibrozil-1-O-\( \beta \)-glucuronide,
CYP2C9 / diclofenac 4'-hydroxylation / tienilic acid, CYP2C19 / (S)-mephentoin 4'-hydroxylation / S-fluoxetine,
CYP2D6 / bufuralol 1'-hydroxylation / paroxetine, CYP2E1 / chlorozoxazine 6-hydroxylation / diethylthiocarbamate,
CYP3A4 / midazolam 1'-hydroxylation / verapamil, diltiazem, and azamulin. Pre-incubation time points were 10 and
30 min. Marker metabolites were quantified by LC/MS/MS.

[Results and Discussion] The IC\(_{50}\) after a 30-min pre-incubation of verapamil was 0.448 \( \mu \)mol/L in the presence of
NADPH, and 23.6 \( \mu \)mol/L in the absence of NADPH. The IC\(_{50}\) shift was 53-fold. We are collecting data for other
time-dependent inhibitors to validate the test system.

IN VITRO ESTIMATION OF P-GLYCOPROTEIN-MEDIATED INTERACTION OF DRUGS WITH
CREMOPHOR EL BY USING DISSOLUTION/PERMEATION SYSTEM
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[Purpose] We have already reported that the Dissolution/Permeation system (D/P system) which can simultaneously
evaluate the drug dissolution and permeation in vitro enables the quantitative assessment of P-glycoprotein-mediated
drug-drug interaction in the oral absorption process. The purpose of this study is to estimate the effect of Cremophor EL,
an ingredient of oral formulation, on the absorption of saquinavir by using the D/P system. [Methods] The D/P system
consists of the apical and basal chambers. Caco-2 monolayers were mounted between both sides. The fasted state
simulated intestinal fluid was used as an apical medium. Saquinavir was applied to the apical side as a solid dosage
form (1/100 of clinical dose) with or without Cremophor EL. Then the dissolved and permeated amounts of saquinavir
were monitored for 2 hrs (% of dose/2 hrs). In vivo oral absorption of saquinavir in humans was estimated from the
correlation between absorption in humans and permeated amount in the D/P system of reference drugs. [Results and
Discussion] In vivo oral absorption of saquinavir (600 mg) was reported to be enhanced approximately 2-fold by
coadministration of Cremophor EL (1000 mg). In the D/P system, the permeated amount of saquinavir in the presence of
Cremophor EL was significantly greater (2.8-fold) than that without Cremophor EL. This difference was larger than the
difference in the dissolved amount (1.7-fold). These results suggested that Cremophor EL, not only facilitated the
dissolution, but also inhibited P-glycoprotein-mediated efflux of saquinavir to enhance its permeation across the
intestinal membrane. In the D/P system, oral absorption of saquinavir was predicted to be enhanced 2-fold by
coadministration of Cremophor EL which corresponds well to the in vivo observation. [Conclusions] The D/P system is
a useful tool to analyze the effect of ingredients in the formulation on the oral absorption of drugs.