CYP PHENOTYPING TO ASSESS DRUG-DRUG INTERACTION

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Phenotyping for drug metabolizing enzymes is defined as measuring its actual in vivo activity in an individual. This is done by administration of a selective substrate for this enzyme and subsequent determination of appropriate pharmacokinetic parameters, or by using metabolism of endogenous substrates. The in vivo effect of a drug on enzyme activity may quantitatively be assessed by comparison of phenotypes in presence and absence of the drug.

Appropriate selection of phenotyping test drugs and metrics is essential to obtain results applicable for other substrates of the respective enzyme. Phenotyping is considered as valid if respective criteria are fulfilled, including (i) changes in metric when patients are treated with inhibitors/inducers of the enzyme; (ii) effect of liver disease if the enzyme is expressed primarily in the liver; (iii) correlation of metabolite formation with activity and content of enzyme in tissue preparations containing the enzyme; (iv) in vitro specificity of the metabolic step; (v) high contribution of the metabolic step to overall drug metabolism; (vi) correlation of metric with the partial clearance for the respective specific metabolic step; (vii) good reproducibility; (viii) correlation of the metric with the AUC of parent substrate; (ix) correlation of the metric with other validated metrics; (x) metric reflects known genetic polymorphism; (xi) metric does not depend on confounding factors such as urinary pH, urinary flow, renal function. Furthermore, the test drug should be registered as a drug, and the procedure should be simple and non-invasive.

Currently, for most of the CYP enzymes important in drug metabolism there are phenotyping procedures which provide valid information on individual activity. The following metrics are recommended based on the level of validation and on practicability (alternative in parentheses): CYP1A2, paraxanthine/caffeine in plasma 6 hours after 150 mg caffeine; CYP2C9, tolbutamide plasma concentration 24 hours after 125 mg tolbutamide ((S)-warfarin plasma AUC after a 10 mg dose of racemic warfarin given in combination with 10 mg of vitamin K to prevent anticoagulation); CYP2C19, urinary excretion of 4'-OH-mephenytoin 0–12 hours after 50 mg mephenytoin; CYP2D6, urinary molar ratio debrisoquine/4-OH-debrisoquine 0–8 hours after 10 mg debrisoquine (dextromethorphan plasma AUC after a 30 mg dextromethorphan-HBr); CYP2E1, urinary ratio 6-OH-chlorzoxazone over chlorzoxazone in plasma 2 hours after 250 mg chlorzoxazone (chlorzoxazone inhibits CYP3A4 and therefore should not be incorporated in phenotyping cocktails); CYP3A4/5, plasma clearance of midazolam after 2 mg midazolam (all drugs given orally). If intestinal and hepatic CYP3A4/5 activities should be assessed separately, hepatic clearance of 1 mg midazolam i.v. may be used for hepatic activity and intestinal availability, calculated from 1 mg midazolam i.v. and 2 mg midazolam orally (sequential administration of use of mass-labelling), is a metric for intestinal CYP3A4/5 activity.

If the effect of a drug on CYP phenotype is to be studied, the extent of intervention should be according to the clinical situation to be assessed. For chronic drug treatment as an intervention, administration of the drug thus should be at the standard therapeutic dose and last until steady state for both the drug (approximately 5 half-lives) and, if any, the changes of enzyme activities is reached. One week appears to be sufficient for enzyme induction. The exposure to the interacting drug should be monitored by quantification of the drug and its relevant metabolites.

Phenotyping procedures may be combined in a »cocktail« to assess a metabolic profile and to simultaneously quantify the effect of a drug on several cytochrome P450 enzymes in vivo. In all cocktails, the selection of metrics is determined by several factors, including the specific objective of the respective study, the availability of analytical methods, and the balance between expense and validation. Many useful cocktails have been reported without mutual interaction between probe drugs and with good tolerability. Still, there is ample space for an improvement by replacement of individual probe drugs, by use of a better validated metric, by reducing the doses, and/or by simplification of the procedures. Low doses and successful validation of limited sampling strategies make the use of cocktails increasingly convenient. The identification of metrics with low intraindividual variability allows the assessment of the effect of interacting drugs with a very limited sample size (12 in most cases). Yes, there is an error in the last sentence, it should be corrected to »In summary, phenotyping and its use drug cocktails is a valuable, safe and scientifically sound tool to quantify the effect of drugs on the in vivo activity of CYP enzymes and thus to assess the metabolic interaction profile of a drug.«