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LC-MS/MS METHOD FOR QUANTITATION OF ALPRAZOLAM, A TYPICAL CYP3A SUBSTRATE, IN PLASMA AND URINE ON IN VIVO DRUG-DRUG INTERACTION STUDY IN CYNOMOLGUS MONKEY
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[Purpose] The development of determination methods for target compounds is imperative for pharmacokinetic studies. Alprazolam (APZ), in contrast to midazolam and simvastatin, is a low-clearance drug in humans and may be a moderate-clearance drug in cynomolgus monkeys, so we considered that the pharmacokinetics of APZ with or without a clinical candidate using cynomolgus monkeys as a preclinical model can predict drug-drug interactions mediated by CYP3A in humans. Thus, we performed the analytical validation for the determination of APZ concentration in cynomolgus monkey plasma and urine using LC-MS/MS on the in vivo induction study of CYP3A.

[Methods] The plasma was deproteinized by the addition of acetonitrile and the diluted supernatant was injected to the LC-MS/MS (APCI method, positive ion mode). A similar analytical validation for the urine was also performed.

[Results and Discussion] The specificity (male and female, n=3), linearity (8 concentration between 0.1 and 100 ng/mL, 1/X², n=3), intraday reproducibility (5 concentration, n=5) and freeze stability (-80°C, 118 days) were confirmed for the plasma and met the acceptance criteria. These validation items also met the acceptance criteria for the urine.

[Conclusions] The analytical method is confirmed to be suitable for determining of APZ concentrations in cynomolgus monkey plasma and urine. In addition, profiles for the plasma concentration and urinary excretion of APZ before and after the induction of CYP3A were obtained.

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EVALUATION OF TIME-DEPENDENT INHIBITION OF DRUG METABOLIZING ENZYMES IN RATS
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Drug interaction caused by the competitive inhibition of metabolizing enzymes could be predicted from the in vitro experiments. While, the quantitative prediction of time-dependent inhibition is still difficult, because the reversibility of enzyme inhibition is not detected in the in vitro experiments which were usually carried out within 1hr. In this study the time-dependent inhibition of CYP was investigated in vitro and compared with the in vivo inhibition profiles. To characterize CYP inhibition, hepatic microsomes isolated from rats were preincubated with inhibitors in the presence of NADPH for 0-30 min. Enzyme activities were assessed by midazolam 1'-hydroxylolation for ketoconazole (KCZ), verapamil (VER), and danazole (DNZ), and by diclofenac 4'-hydroxylation for cimetidine (CIM). Except for KCZ, 30 min-preincubation in the presence of NADPH potentiated the inhibitory effect. The results suggested that these drugs except for KCZ inhibited the CYP activities in a time-dependent manner. To test the reversibility of the inhibition, microsomes was incubated with these drugs in the presence of NADPH for 30min and then dialyzed at 4°C. After 16hr dialysis, the inhibitory effect of KCZ, VER and CIM was decreased, but the effect of DNZ did not changed, indicating that the inhibition of KCZ, VER and CIM were reversible, but that of DNZ was irreversible. The enzyme activities in liver after administration of these drugs were also determined to evaluate the in vivo effect of the CYP inhibitors. After administration of 100mg/kg of CIM, the enzyme activities were 68% of control values at 6hr, and recovered to 97% at 18hr. The recovery was much faster than expected from the reported turnover rate of the enzyme. This could be explained by the result that the inhibition is not irreversible. In the present findings indicated that reversibility of inhibition must be taken into consideration for accurate prediction of time-dependent drug inhibition.