Establishment of CYP induction screening using cryopreserved human hepatocytes

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Cryopreserved human hepatocytes were evaluated for use in CYP induction screening as they are readily available and can be assayed quickly with a small sample size. We have previously reported the evaluation of CYP induction using a 24-well plate. In this study, we attempted to further improve CYP induction screening at the drug discovery stage by increasing throughput. After a 3-day pre-culture, the hepatocytes were exposed to the evaluation compounds for 3 days and CYP activities were measured. Concentration dependencies of CYP3A and CYP1A activities were observed on 3 lots of hepatocytes. The culture conditions allowed detection of the concentration dependency of not only phenobarbital (PB) and rifampicin (RIF) but also weak CYP3A inducers carbamazepine and phenytoin. For the enzyme activity measurement, introducing UPLC (CYP3A) and a fluorescence plate reader (CYP1A) provided a noticeable improvement in throughput quantitative analysis. We assayed 18 compounds within 7 days from cell seeding to measurement of activity by using 96-well plates [6 plates/vial (1A+3A), 6 cmpds/plate, 3 concentrations/cmpd], whereas the evaluation of only 2–3 compounds is possible with a conventional 24-well plate. Although a large variation of activities (expressed as fold of control) between the 3 lots of hepatocytes was observed, variation in the correction values, using the activities of omeprazole (OMP, CYP1A) and RIF or PB (CYP3A) as the positive control factors was small. By setting OMP, RIF or PB as the positive control for every test and calculating the correction value for each compound, continual screening evaluation is possible even with different lots of cryopreserved human hepatocytes. We concluded the criteria for judging high risk of induction capacity to be >30 µmol/L OMP for CYP1A and >200% of control for CYP3A.

Prediction of the effect of genetic polymorphisms of CYP2C9 on the pharmacokinetics of substrate drugs from in vitro data

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CYP2C9 has some polymorphic alleles (especially *2 and *3) and the examples of the inter-individual variability of pharmacokinetics of drugs metabolized by CYP2C9 have been documented in the clinical situation. The previous reports indicated that the percent reduction of the clearance of subjects with polymorphic alleles compared with wild type alleles varies among individual drugs. The substrate specificities of the effects of polymorphisms on the pharmacokinetics are caused not only by the difference in the percent reduction of intrinsic clearance normalized by expression level but also by other factors such as the difference in the contribution of CYP2C9 to the total clearance. However, nobody has investigated whether the changes in total clearance of CYP2C9 substrates were quantitatively predicted from the in vitro experimental data or not. We collected the literature information about the contribution of CYP2C9-mediated metabolism to the overall clearance and in vitro experimental data using mutated CYP2C9 and predicted the in vivo reduction of the clearance in subjects with several kinds of CYP2C9 diplotypes. We found that the percent reduction of clinically-observed total clearance of each substrate for each diplotype was well correlated with that predicted from in vitro data and all data points were approaching to 1:1 correlation line compared with the in vitro data without considering the contribution of CYP2C9. This result indicated that the correct estimation of the contribution of a target enzyme to the overall clearance is necessary for the accurate prediction from in vitro data.