THE LONG-TERM MAINTENANCE OF METABOLIC ACTIVITIES IN 3-DIMENSIONAL CULTURE USING RAT HEPATOCYTES HOLLOW FIBERS

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When hepatocytes are packed into the lumen of hollow fibers by a centrifugal filling procedure and are cultured in 3-dimensions (3D), hepatocyte organoid formation is facilitated. Using this technique, hepatic functions, such as albumin production and ammonia metabolism, can be maintained over the long term. The TESTLIVER-Rat- is a commercially available version of the rat hepatocyte-filled hollow fibers that is readily obtainable from TOYOBO. We examined the time courses of cytochrome P450 (CYP1A, 2B, 2C, and 3A) and conjugation (glucuronidation and sulfation) activities for 28 days in the TESTLIVER-Rat-. In the current study, CYP1A, 2B, 2C, and 3A activities were simultaneously measured using a cocktail of 7-ethoxyresorufin (for CYP1A) and testosterone (for CYP2B, 2C9, and 3A) as probe substrates; the conjugation activities were simultaneously measured using 7-hydroxycoumarin as a probe substrate. After receipt of the TESTLIVER-Rat- from TOYOBO, the time courses of the CYP activities were measured. It was demonstrated that individual CYP activity decreased by only 20% during culture period. The sum of each CYP activity in the TESTLIVER-Rat- was approximately one-third of that measured immediately after the preparation of cryopreserved rat hepatocytes. It became clear that this culture system is both easy to manipulate and maintains high CYP activities under long-term culture conditions. Thus, the 3D-culture of hepatocytes using hollow-fibers may find an application in DMPK studies such as the preparation of large amounts of metabolites and the elucidation of CYP induction. Currently, we are using the TESTLIVER-Rat- to investigate the time courses of conjugation activities.

IDENTIFICATION OF HUMAN CYTOCHROME P450 ISOZYMES INVOLVED IN DIPHENHYDRAMINE N-DEMETHYLATION

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Diphenhydramine (DPHM) is widely used as an over-the-counter antihistamine. In this study, the specific human cytochrome P450 (P450) isozymes involved in N-demethylation, a main metabolic pathway of DPHM, were identified in a range of clinically relevant concentrations (0.14 to 0.77 µM) by an LC/MS method developed in our laboratory. Among 14 recombinant P450 isozymes, CYP2D6 showed the highest level of activity of DPHM N-demethylation (0.69 pmol/min/pmol P450) at 0.5 µM. CYP2D6 catalyzed DPHM N-demethylation as a high-affinity P450 isozyme, the Km value of which was 1.12 ± 0.21 µM. In addition, CYP1A2, CYP2C9 and CYP2C19 were identified as low-affinity components. In human liver microsomes, involvement of CYP2D6, CYP1A2, CYP2C9 and CYP2C19 in DPHM N-demethylation was confirmed by using P450-isozyme specific inhibitors. In addition, contributions of these P450 isozymes estimated by the relative activity factor were in good agreement with the results of inhibition studies. Although an inhibitory effect of DPHM on the metabolic activity of CYP2D6 has been reported previously, the results of the present study suggest that it is not only a potent inhibitor but also a high-affinity substrate of CYP2D6. Therefore, it is worthy to mention that the sedative effect of DPHM might be caused by co-administration of CYP2D6 substrate(s)/inhibitor(s). In addition, large differences in the metabolic activities of CYP2D6, and those of CYP1A2, CYP2C9 and CYP2C19 could cause the individual differences in anti-allergic efficacy and sedative effect of DPHM.