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24-HR RHYTHM OF CYP2E1 ACTIVITY IN MOUSE LIVER AND ITS POSSIBLE MECHANISM
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Cytochrome (CYP) P450 2E1 is clinically and toxicologically important. Because CYP2E1 metabolizes a wide variety of chemicals with different structure, in particular small and hydrophobic compounds, including potential cytotoxic and carcinogenic agent. In addition, circadian rhythm is observed in drug metabolism in liver. In this study, we investigated whether the liver transcription factor HNF-1α (hepatic nuclear factor) and clock genes undergoing a striking 24-hr rhythm in mouse liver contribute to the 24-hr regulation of CYP2E1 activity. Male ICR mice (7 weeks old) were purchased from Charles River Japan Inc. They were housed under a standardized light/dark cycle at room temperature of 24 ± 1°C and a humidity of 60 ± 10% with food and water ad libitum for 2 weeks. Liver was obtained at 09:00, 13:00, 17:00, 21:00, 01:00 or 05:00. CYP2E1 activity was determined by measuring p-nitrophenol (PNP)-hydroxylase. The amount of protein and mRNA was measured by Western blot analysis and RT-PCR. CYP2E1 promoter activity was measured by Dual-Luciferase assay. CYP2E1 activity in the liver exhibited a significant 24-hr rhythm. CYP2E1 activity increased during dark-phase, reaching its highest level between 21:00 and 01:00. The protein levels of CYP2E1 also increased during dark-phase. Namely, 24-hr rhythm CYP2E1 activity corresponded to that of protein amount. In addition, the mRNA levels of CYP2E1 rose sharply during afternoon and reached maximum at 17:00. By considering the results of luciferase assay, 24-hr rhythm of CYP2E1 activity was suggested to be controlled by a transcription level. Significant 24-hr rhythmicity was demonstrated for CYP2E1 activity, protein levels and mRNA levels. HNF-1α and clock genes contribute to produce the 24-hr rhythm of CYP2E1 mRNA levels.

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CYP3A4 INDUCTION IN SPHEROIDAL HepG2 CELLS FORMED ON A 96-WELL NANO CULTURE PLATE
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CYP3A4 plays a major role in the metabolism of wide variety of therapeutic drugs. CYP3A4-based drug-drug interaction is often concerned. Drug-drug interaction through CYP3A4 induction by rifampicin is a well-known example. In consideration of problems in availability of high-quality primary human hepatocytes, we attempted to develop detection system of CYP3A4 induction in a spheroid-culture system of a HepG2 cell on 96-well “Nano Culture Plate” (SCIVAX). We also cultured HepG2 cells on an ordinary 96-well cell culture plate as a reference (2D culture). Refampicin was added at 0 time or 48hr after inoculation, and further cultured for 48 or 72 hrs. Total RNA was extracted, reverse-transcribed, and subjected to real-time polymerase chain reaction to measure mRNA levels of CYP3A4, CYP3A5, CYP1A2, UGT1A1, pregnane X receptor (PXR), and glucocorticoid receptor (GR). Overall, no appreciable difference in GAPDH expression was observed between in the 2D culture and the spheroid system. CYP3A4, CYP3A5, CYP1A2 and UGT1A1 were induced by rifampicin in the 2D culture as well as in the spheroidal cells: HepG2 cells used in the present study showed good response in terms of CYP3A4 induction by rifampicin even in the 2D culture. No appreciable induction of PXR was observed either in 2D culture or in spheroids. Induction of CYP3A4, CYP3A5, CYP1A2 and UGT1A1 by “known CYP3A4 inducers” namely, paclitaxel, clotrimazole, simvastatin, phenytoin and valproic acid, are under evaluation. Induction of CYP3A-associated enzyme activity will also be presented.