DRUG METABOLISM RELATED GENE EXPRESSION PROFILES IN PHENOBARBITAL OR β-NAPTHOFLAVONE ADMINISTERED RAT LIVER, KIDNEY AND SMALL INTESTINE USING 24 SAMPLES ANALYZABLE DNA MICROARRAY

Isao Miyagawa¹, Atsushi Morishita¹, Kazuyuki Yanagibashi¹, Kazuhiko Kogoh²
¹Technical Research Laboratory, Kurabo Industries Ltd. 14-5 Shimokida-cho, Neyagawa, Osaka 572-0823 and ²New Business Development Department, Kurabo Industries Ltd. 4-31 2-chome, Kyutaro-machi, Chuo-ku, Osaka 541-8581, Japan

Drug metabolism related gene expression analysis is important to avoid drug-drug interaction at the early stage of drug discovery. The mRNA expression of CYP1A and CYP3A genes are commonly measured. It is also important to measure expression of other CYP, transferase, transporter and nuclear receptor genes and moreover to analyze the dose- or time-dependent behaviors. However, such comprehensive analysis requires lots of work. In this study, we developed the new DNA microarray which can analyze 24 samples at one time and analyzed the dose-dependent expression profiles of 115 drug metabolism related genes in rat liver, kidney and small intestine. Five weeks old male Sprague-Dawley rats were intraperitoneally administered 1mg, 10mg or 100mg/kg/day of Phenobarbital (PB) or β-Napthoflavone (NF) for 3days (n=2). Total RNA of 48 samples were isolated from collected liver, kidney and small intestine. We measured mRNA expressions using two DNA microarrays and detected the expression of 84 genes in liver, 54 genes in kidney and 51 genes in small intestine. In liver tissue, 20 genes (e.g. Cyp2b1,2, Ugt2b1, Mrp3) were upregulated in PB dose-dependent manner and 13 genes (e.g. Cyp1a1,1a2, 1b1, Ugt1a families, Gstp1, 2) were also upregulated in NF dose-dependent manner. Several genes were also upregulated dose-dependently in kidney and small intestine. The gene expression profiles in female rat tissues will also be discussed.

REGULATION OF Cyp3a41 AND Cyp3a44 GENE EXPRESSION MEDIATED BY GLUCOCORTICOID RECEPTOR

Wattanaporn Bhadhprasit, Tsutomu Sakuma, Masahiro Fuwa, Shin-ichi Ueno, Kaori Kitajima and Nobuo Nemoto
Graduate school of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

Although the glucocorticoid receptor (GR) facilitates the xenobiotic-induced expression of CYP3A in rodents, its role in the regulation of female-specific CYP3A41 and CYP3A44 is not clearly understood. In this study, the role of mouse GR in the regulation of CYP3A41 and CYP3A44 was evaluated using primary cultured mouse hepatocytes. Dexamethasone (DEX) can induce the expression of Cyp3a41 gene via a GR-dependent mechanism. However, ecteinascidin-743 (ET-743), a pregnane X receptor (PXR)-antagonist, could not antagonize DEX-induced CYP3A41 expression. This finding suggests that the PXR may not play an important role in the regulation of Cyp3a41 gene. Sequence analysis of the 5’-flanking region (-163/+61) of Cyp3a41 gene revealed the existence of the putative binding sites for HNF4, PXR or GR. However, reporter gene assays revealed that only HNF4 binding site is functional for the cis-activation of Cyp3a41 -163/+61 enhancer. DEX induces CYP3A44 mRNA expression in a biphasic manner depending on DEX-concentrations, which suggests that the expression may be mediated via the pathways involving either a low- or a high-concentration component. In the low-concentration component (at submicromolar concentrations), DEX regulates through the GR pathway, whereas the high-concentration component (at supramicromolar concentrations), the expression is mediated through DEX-mediated PXR activation. Overall, these results indicate that different mechanisms are involved in the regulation of Cyp3a41 and Cyp3a44 gene expression. This study deals with the role of GR in the overall regulation of mouse Cyp3a41 and Cyp3a44 gene expression.