30PE-09

EFFECTS OF INTESTINAL MICROBIOTA ON THE EXPRESSION OF CYP ENZYMES
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We have previously shown a possibility that the expression of hepatic Cyp3a is increased by the intestinal microbiota. The present study has been undertaken to investigate its effect on other CYP isozymes together with the alteration mechanism. Real-time RT-PCR analyses indicated that the hepatic mRNA levels of Cyp1a2, Cyp2a4, Cyp2b9, Cyp2b13, Cyp2c37, Cyp3a11, Cyp3a25, Cyp3a16, Cyp3a41 and Cyp3a44 were significantly higher in the specific pathogen free (SPF) mice than in the germfree (GF) mice. In order to elucidate the mechanism of CYP induction, the expression of hepatic nuclear receptors which regulate the CYP expression was investigated. As a result, hepatic mRNA levels of the farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR) were significantly elevated in the SPF mice compared with the GF mice. The SPF mice also showed a higher expression of the hepatic transporters (Oatp2, Oct1, Ntcp and Mrp3) and the conjugating enzymes (PAPSS2 and Sult1d1) which are involved in the detoxification of lithocholic acid (LCA) produced by the intestinal microbiota. Because LCA is known as a ligand of FXR and PXR, it is possible that the induction of hepatic CYP enzymes in the SPF mice results from the activation of these nuclear receptors by LCA.

The composition of the intestinal microbiota is known to be changed by drugs, ageing, diseases etc. The findings in the present study suggest the possibility that the alteration of the intestinal microbiota might contribute to the intra- and inter-individual variability of pharmacokinetics.

30PE-10

5'-REGULATORY REGION FOR TRANSCRIPTIONAL ACTIVATION OF THE MOUSE CYPIA2 GENE INDUCED BY 3-METHYLCHOLANTHRENE AND 2,3,7,8-TCMCHLORODIBENZO-P-DIOXIN
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CYP1A2 is one of the major cytochrome P450s constitutively expressed in mammalian liver. Hepatic CYP1A2 is inducible by polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene (3-MC), and by hepatocarcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We have previously reported two enhancer elements, existing in the region 4.8 kilobases upstream from the transcription start site of the mouse Cyp1a2 gene, which regulates its constitutive expression. However, the mechanism regulating transcriptional activation of Cyp1a2 by 3-MC and TCDD is still unclear. Here we examined the region responsible for the mouse Cyp1a2 expression induced by 3-MC and TCDD, using reporter gene assay system. Hepatocytes from C57BL/6 mice in primary culture were transfected with the reporter plasmid. The -4.8 kbp/pGL3-basic, which includes three putative xenobiotic responsive elements (XREs), did not show significant increased reporter activity following exposure to 3-MC or TCDD, compared with that of cells transfected with pGL3-basic empty vector. In the next experiment, a further 5'-upstream region from -12,415 to -13,965, which includes twelve XREs was ligated to the -4.8 kbp/pGL3-basic. The reporter activities were increased 2-fold by 3-MC and 6-fold by TCDD, respectively. Furthermore, the region including twelve XREs, from -12,527 to -13,965, was subcloned into pGL3-promoter, and the reporter activities were increased 8-fold by 3-MC and 20-fold by TCDD, respectively. AhR antagonist, α-naphthoflavone, inhibited the transcriptional activation induced by TCDD. These findings suggest that the transcriptional activation of the mouse Cyp1a2 induced by 3-MC and TCDD is regulated via the 5'-upstream region from -12,527 to -13,965 that includes twelve XREs.