

Rapid Communication

Monitoring Expression of Cytochrome P450 Genes During Postischemic Rat Liver Reperfusion Using DNA Microarrays

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Abstract. We investigated the expression of 50 cytochrome P450 (CYP) genes in the post-ischemic reperfused rat liver using a DNA microarray. Thirteen CYPs showed absent expression in all experiments and 2 CYPs were induced by pentobarbital, which was used as an anesthetic. Eight of the remaining 35 CYPs showed significantly decreased expression following ischemia-reperfusion. Monitoring CYP expression may be a powerful approach for elucidation of pathways regulating drug metabolism that may be involved in postischemic reperfusion liver injury.

Keywords: cytochrome P450, postischemic liver reperfusion, gene expression

The cytochromes P450 (CYPs) constitute a super-family of enzymes that play an important role in the oxidative metabolism of numerous endogenous and foreign chemicals (1). Investigation of the expression profile of many CYP genes in pathological disorders resulting in a disease may help elucidate the underlying mechanism for regulation of the transcription of CYP genes in the process of pathogenesis and also predict drug metabolism and toxicity in a disease. In pathological disorders caused by postischemic reperfusion injury, induction and repression of some CYP metabolic activity have been described in the rat liver (2). However, the expression profile of CYP genes during postischemic liver reperfusion is not yet clear. In this report, we investigated the expression pattern of 50 CYP genes in the rat liver during postischemic reperfusion using a DNA microarray (GeneChip; Affymetrix, Santa Clara, CA, USA).

Male Wistar-Kyoto rats (Shizuoka Laboratory Animal Center, Hamamatsu), weighing 300 to 400 g, were anesthetized with pentobarbital (50 mg/kg, i.p.). Liver ischemia was induced by clamping the portal vein and hepatic artery for 15 min as previously described (3). Then, the liver was allowed to reperfuse for various times. Sham operation consisted of laparotomy and liver manipulation under pentobarbital anesthesia. All

animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences. To analyze the pattern of CYP gene expression in response to the stress of ischemia-reperfusion in the rat liver, RNA was isolated from liver samples at 0, 15, 30 min and 1, 2, 4, 8, 16, and 32 h (9 time points) after ischemic reperfusion and sham control at 0, 8, and 16 h (3 time points). RNA analysis was performed ($n=3$ for each time point) using an Affymetrix Rat Toxicology U34 array as previously described (4). In this array, we selected 79 probes for 50 CYP genes (see <http://www.affymetrix.com/analysis/index.affx> and <http://drnelson.utmem.edu/CytochromeP450.html>). Thirteen CYP genes were assigned as “absent” by Affymetrix GeneChip software in all hybridizations and were eliminated from the data set. In addition, 2 CYP genes (CYP2B1 and CYP2B2) were also eliminated because their expression significantly differed at 8 or 16 h in the sham control compared with 0 h in the sham control ($P<0.05$, Welch’s t -test). Because various phenobarbital-like inducers in addition to phenobarbital reportedly induce both CYP2B1 and CYP2B2 (5), we further examined the effect of pentobarbital on the expression of CYP2B genes by TaqMan RT-PCR, as previously described (6). Figure 1A showed that both CYP2B1 and CYP2B2 gene expression were markedly increased in the pentobarbital-treated liver compared

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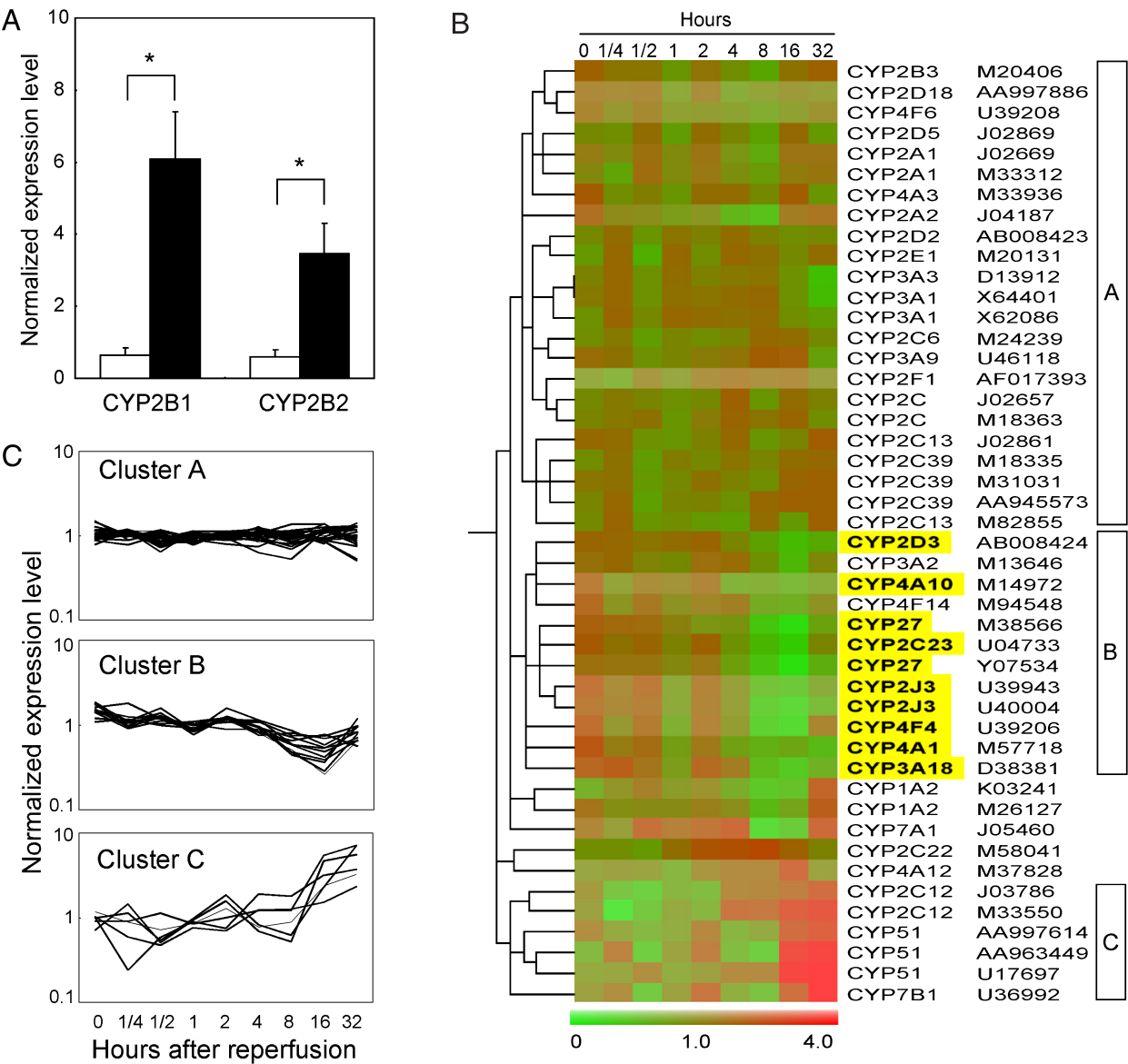


Fig. 1. TaqMan RT-PCR assay showing CYP2B induction by pentobarbital (A) and clustering analysis of gene expression pattern (B) and average expression profiles (C) of 35 CYP genes generated by GeneChip analysis. A: CYP2B1 and CYP2B2 gene expression in the rat liver were significantly increased at 8 h after pentobarbital treatment (closed bars) compared to the saline control (open bars) (* $P<0.05$, Welch's t -test). Data represent the mean \pm S.E.M. ($n = 5$ in each assay). B: Hierarchical clustering and expression of 35 CYP genes using a 46-probe set during postischemic-reperfusion with a class label indicating the cluster (A through C). Time after reperfusion is shown on the horizontal axis. Common names and GenBank numbers are shown on the vertical axis. Yellow highlighting indicates a gene that showed significantly different expression compared with 0 h after reperfusion ($P<0.05$, Welch's t -test). C: Average expression profiles for each of the three sets obtained by hierarchical clustering. The class label of each CYP gene is indicated in Fig. 1B.

with the saline controls ($P<0.05$). This result indicates that the expression of CYP2B genes would have been influenced by pentobarbital used as an anesthetic.

The remaining 46 probes corresponding to 35 CYP genes were further analyzed. Hierarchical clustering by standard correlation was performed using GeneSpring software (Silicon Genetics, Redwood, CA, USA). The expression pattern of these 35 CYP genes was broadly

clustered into three groups using hierarchical clustering (Fig. 1B). Average expression profiles for each of the three sets obtained by hierarchical clustering are shown in Fig. 1C. Cluster A consisted of 23 probes for 17 genes showing stable or slightly different expression during reperfusion. This set included the CYP2A, CYP2C, and CYP3A families. Cluster B consisted of 12 probes for 10 genes showing decreased expression between 8 to 16 h

of reperfusion. Cluster C consisted of 3 genes (6 probes) showing induction after 16 h of reperfusion. This set included CYP2C12, CYP7B1, and CYP51. However, these 3 CYPs in cluster C showed no statistically significant change in expression after reperfusion (Fig. 1B).

Among 35 CYPs, only 8 genes showed significantly different expression at any time point after reperfusion compared with 0 h after reperfusion ($P < 0.05$, Welch's *t*-test), as highlighted in Fig. 1B. All of these 8 CYP genes showed significant down-regulation at 16 h after reperfusion, being classified into cluster B. To validate our microarray results, we selected CYP4A1 and CYP27 for TaqMan RT-PCR for semiquantitative measurement of relative RNA abundance. Figure 2 shows that there was excellent concordance of the results of TaqMan RT-PCR and GeneChip findings. CYP4A1 and CYP2C23, which have arachidonic acid monooxygenase and epoxigenase activity, respectively, play an important role in arachidonic acid metabolism. Decreased mRNA

and protein expression of CYP4A1 and decreased level of CYP2C23 following ischemia-reperfusion have been shown in the rat kidney (7, 8). Also, the mRNA expression of CYP4F4, which catalyzes the metabolism of leukotrienes and prostaglandins, was reportedly decreased in the rat hippocampus at 24 h following traumatic brain injury (9). In this study, CYP4A1, CYP2C23, and CYP4F4 gene expression in the rat liver were significantly decreased following ischemia-reperfusion. These observations together with previous reports suggest that arachidonic acid metabolism may be involved not only in the kidney but also in the liver during postischemic reperfusion. Supporting this, prostaglandins and synthetic prostaglandin analogs have shown cytoprotective effects on hepatocytes in experimental models of ischemia-reperfusion liver injury and treatment with prostaglandins is reported to improve graft function after rat liver transplants (10). In addition, we found that the mRNA expression of CYP27, the sterol 27-hydroxylase, which is a rate-limiting enzyme for bile acid synthesis, was decreased following ischemia-reperfusion. This observation is consistent with the fact that liver failure and hepatic dysfunction are associated with a reduction of bile acid synthesis. Thus, monitoring the expression of many CYPs using DNA microarrays may help to provide an overview of which metabolizing pathways are involved and a prediction of which drugs cause potential side effects or toxic insult during postischemic rat liver reperfusion.

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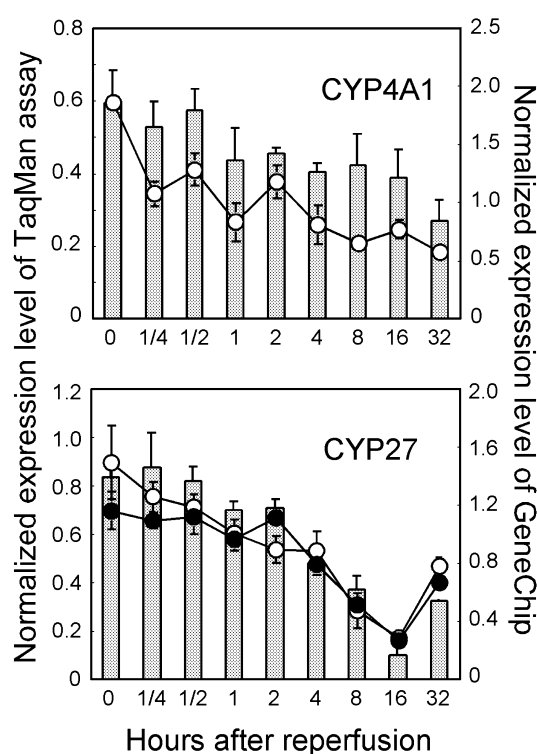


Fig. 2. Validation of microarray results by TaqMan RT-PCR assay. Relative expression levels of two selected genes (CYP4A1, CYP27) were measured by TaqMan RT-PCR assay (bars) in comparison to GeneChip (lines). Quantitation with the two methods gave very similar results. Data represent the mean \pm S.E.M. in TaqMan RT-PCR ($n = 5$) and mean in microarray ($n = 3$). Microarray data were re-normalized as recommended in the GeneSpring software. GeneChip probes used in this graph were M57718mRNA_s at for CYP4A1 and M38566mRNA_s at (open circles) and Y07534cds_s at (closed circles) for CYP27.

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