Dietary Cholesterol Fails to Stimulate the Human Cholesterol 7α-Hydroxylase Gene (CYP7A1) in Transgenic Mice*§

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Dietary cholesterol has been shown to have a stimulatory effect on the murine cholesterol 7α-hydroxylase gene (Cyp7a1), but its effect on human cholesterol 7α-hydroxylase gene (CYP7A1) expression in vivo is not known. A transgenic mouse strain harboring the human CYP7A1 gene and homozygous for the disrupted murine Cyp7a1 gene was created. Cholesterol feeding increased the expression of the endogenous modified Cyp7a1 allele but failed to stimulate the human CYP7A1 transgene. In transfected hepatoma cells, 25-hydroxycholesterol increased murine Cyp7a1 gene promoter activity, whereas the human CYP7A1 gene promoter was unresponsive. Electrophoretic mobility shift assays demonstrated the interaction of the liver X receptor α (LXRα); retinoid X receptor (RXR) heterodimer, a transcription factor complex that is activated by oxysterols, with the murine Cyp7a1 gene promoter, whereas no binding to the human CYP7A1 gene promoter was detected. The results demonstrate that the human CYP7A1 gene is not stimulated by dietary cholesterol in the intact animal, and this is attributable to the inability of the CYP7A1 gene promoter to interact with LXRαRXR.

Bile acids represent the terminal end products of cholesterol catabolism. Bile acid synthesis takes place exclusively in the liver, and the pathways responsible for this synthesis of the bile acids in the enterohepatic circulation is controlled by a microsomal enzyme known as cholesterol 7α-hydroxylase (cyp7a).1 The activity of cyp7a is regulated by a variety of nutritional and hormonal factors involving both feedback and feedforward mechanisms. The regulatory control appears to be exerted mainly at the level of gene transcription, since cyp7a activity is highly correlated with cyp7a mRNA abundance.

Much of what is known about the regulation of cyp7a gene expression has come from studies involving animal models. It has been known for a long time that cyp7a gene expression in rodents is repressed by bile acids and stimulated by cholesterol. The inhibition of cyp7a gene expression by bile acids is accomplished via an indirect mechanism that involves a nuclear factor that interferes with gene transcription (3–5). The stimulation of cyp7a gene expression by cholesterol is mediated through liver X receptor (LXRα), an oxysterol-activated nuclear receptor that binds to the cyp7a gene promoter as a heterodimer with another nuclear receptor known as retinoid X receptor (RXR) (6–8).

Very little is known regarding the regulation of the gene encoding the human cyp7a (CYP7A1), particularly under non-pathological conditions. In this report, we describe the creation of a new mouse model that uniquely expresses the human CYP7A1 gene in the liver. We used this model to evaluate the response of the human CYP7A1 gene to regulation by dietary cholesterol in the intact animal, in an effort to gain insight into the mechanisms that regulate human CYP7A1 gene expression in vivo.

EXPERIMENTAL PROCEDURES

Mice—Transgenic mice were produced by injecting an entire bacterial artificial chromosome (∼125 kb) containing the human CYP7A1 gene into C57BL/6 mice. The resulting F1 offspring were interbred to produce F2 CYP7A1 transgenic mice that were also homozygous for the disrupted Cyp7a1 allele and a primer pair (IMR594, 5′-GATGTATGC-3′ and IMR595, 5′-TCACAAGGTGCGTCTTAGCC-3′) that is specific for mouse genomic DNA. Transgenic mice were crossed with C57BL/6J mice, and the resulting F1 generation was analyzed for transgene inheritance and expression. The human CYP7A1 gene was detected by RNA blotting using the rat cyp7a cDNA as a probe. The human cyp7a mRNA was detected in total liver RNA (see below for details), and the human cyp7a enzyme was detected by immunoblotting using rabbit antiserum directed against the last five carboxyl-terminal amino acid residues of human cyp7a. The CYP7A1 transgenic mouse line in which the human cyp7a mRNA and enzyme could be detected unequivocally was expanded. A male transgenic mouse from the F2 generation was used to breed the CYP7A1 transgene into female Cyp7a1−/− mice obtained from The Jackson Laboratory (Bar Harbor, ME). The F1 progeny of this cross were interbred to produce F2 CYP7A1 transgenic mice that were also homozygous for the disrupted Cyp7a1 allele. Zygosity to the mutant Cyp7a1 allele was evaluated by PCR analysis using a primer pair (IMR013, 5′-CTTGGGTGGAGAGGGTTATTCTTCTCTC-3′; IMR014, 5′-TGACATCTGATCACTACGACACT-3′) specific for the neomycin phosphotransferase gene (neo’) that is embedded in the disrupted Cyp7a1 allele and a primer pair (IMR394, 5′-GATGTATGC-3′; IMR595, 5′-TCACAAGGTGCGTCTTAGCC-3′) specific for a segment of the wild type Cyp7a1 gene that is absent in the mutant Cyp7a1 allele. The IMR primers described above are docu-

1 The abbreviations used are: cyp7a, cholesterol 7α-hydroxylase; LXRα, liver X receptor α; RXR, retinoid X receptor; RT, reverse transcriptase; CAT, chloramphenicol acetyltransferase.

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Human CYP7A1 Gene Expression in Transgenic Mice

Introduction

Cyp7a1 mice were generated by inserting a LXR binding site into the Cyp7a1 locus in a transgenic mouse model. The transgenic mice were generated by using a LightCycler software package (Roche Diagnostics Canada). Cyclophilin mRNA was used as the internal standard, and its abundance was determined using a standard curve. The results were expressed as a percentage of the total amount of mRNA present in each sample.

RESULTS AND DISCUSSION

We obtained nine mice from injected fertilized eggs that had incorporated the transgene into their genomes, and five of these transmitted the transgene to their offspring (see Supplemental Table I for details). The human cyp7a mRNA was detectable in only three of the five lines. One of these lines produced very small litters and was not bred further. The human cyp7a mRNA and enzyme (Fig. 1, A and B, respectively) were unequivocally detectable in only one (line M49) of the two remaining lines, and this was selected to establish a colony. The F3 generation was produced by crossing two hemizygous transgenic mice.

The human CYP7A1 transgene was subsequently transferred to the Cyp7a1+/− background by crossing a transgenic male mouse from the F3 generation with female Cyp7a1+/− mice. The progeny of this cross were heterozygous for the disrupted Cyp7a1 allele, and all contained the human CYP7A1 transgene. As shown in Fig. 2A, both the human CYP7A1 gene and the disrupted murine Cyp7a1 gene hybridization patterns (left and right lanes, respectively) are evident in the progeny of this cross (middle lane). A male and female mouse from this generation were mated to reestablish homozygosity to the disrupted Cyp7a1 allele. From the progeny of this cross, one male and one female mouse, which carried the desired genotype, were selected to establish the second colony. Fig. 2B shows the genotypic analysis of Cyp7a1+/− mice carrying the human CYP7A1 transgene (hereafter referred to as hCYP7A1 mice).

As can be seen (Fig. 2B, lane 5), the human CYP7A1 transgene is clearly detectable, and the wild type Cyp7a1 allele has been replaced with the disrupted Cyp7a1 allele. Expression of the human CYP7A1 transgene in Cyp7a1+/− mice reversed postnatal lethality observed in Cyp7a1+/− mice (13). The hCYP7A1 pups were overtly indistinguishable from wild type pups and survived to weaning without supplementing the standard diet of nursing mothers with bile acids or fat-soluble vitamins.

Liver expression of the CYP7A1 transgene in mice possessing the wild type Cyp7a1 gene was reported earlier (14), but the tissue distribution was not determined. Thus, we surveyed the distribution of CYP7A1 transgene expression in hCYP7A1 mice. As shown in Fig. 2C, the human cyp7a mRNA is detectable in liver tissue only (black arrow). This result demonstrates that the liver-specific expression of the human CYP7A1 gene is faithfully reproduced in the murine species.

The stimulation of the murine Cyp7a1 gene expression by dietary cholesterol is well documented (8, 15, 16). Thus, we compared the expression of the CYP7A1 transgene in mice fed the standard chow diet or the chow diet supplemented with 1% cholesterol for 14 days. Although the Cyp7a1−/− mice do not synthesize the cyp7a enzyme, the disrupted murine Cyp7a1
allele continues to be transcribed producing an mRNA species that is a composed of cyp7a and neo' sequences (13). The changes in the abundance of the chimeric murine cyp7a/neo' mRNA in response to cholesterol feeding were also monitored. As shown in Fig. 3A, the murine cyp7a/neo' mRNA was increased by 40% (p < 0.0005) in hCYP7A1 mice. This rise in cyp7a/neo' is in agreement with the expected response of the murine Cyp7a1 gene to dietary cholesterol. In contrast, the abundance of the human cyp7a mRNA was decreased by 56% (p < 0.005). The Abca1 gene is expressed in the liver (17) and is known to be stimulated by oxysterols (18). A 7.8-fold rise (p < 0.006) in Abca1 mRNA abundance in total liver RNA of cholesterol-fed versus chow-fed hCYP7A1 mice was evident (Fig. 3B). Although it is not clear which hepatic cell type expresses the Abca1 gene, the result is nevertheless consistent with the exposure of the liver of cholesterol-fed mice to the dietary cholesterol. The hepatic cyp7a enzyme activity of chow-fed hCYP7A1 mice is lower than that of chow-fed wild type mice, which may be due to the lower level of human CYP7A1 transgenic expression relative to that of the murine Cyp7a1 gene in wild type mice. The cholesterol diet decreased hepatic cyp7a enzyme activity by 55% (p < 0.02) in hCYP7A1 mice, concomitant with the fall of human cyp7a mRNA abundance (Fig. 3C). These results clearly illustrate the failure of dietary cholesterol to stimulate human CYP7A1 transgene expression in mice. The apparent repression of human CYP7A1 gene expression by dietary cholesterol was unexpected, and the basis for this is not clear. It is evident that, in intact animals, the human CYP7A1 and murine Cyp7a1 genes respond differently to dietary cholesterol.

The findings obtained in vivo were verified in hepatoma cells. It is known that oxysterols regulate the activity of responsive promoters by activating nuclear receptors known as the LXR, which are bound to response elements as heterodimers with RXRs (6). The murine Cyp7a1 gene promoter contains an LXR: RXR binding site (termed Site I; Fig. 4A) (11), which is similar in sequence to the rat Cyp7a1 LXR binding site (19). In addition, the inactivation of the murine gene encoding the LXRα isoform results in the resistance of the Cyp7a1 gene to stimulation by dietary cholesterol (8). Thus, we compared the response of the human and murine gene promoters to oxysterol-activated LXRα:RXR. Fig. 4B shows a 30-fold increase (p < 0.001) in reporter activity from the murine mCyp7a1.CAT gene chimera when LXRxα was activated by 25-hydroxycholesterol,
whereas no response was evident from the human hCYP7A1.CAT gene chimera. A similar finding for the human CYP7A1 gene promoter was also documented in HepG2 cells (19). Analysis of recombinant LXRα:RXR interaction with human and murine Site I by electrophoretic mobility shift assay demonstrated avid binding of the heterodimer to the murine Site I, but no detectable binding to the human Site I was found (Fig. 4C). Moreover, the binding of LXRα:RXR to the murine Site I could be efficiently competed by an idealized LXRα:RXR binding site, illustrating the specificity of the interaction. These results demonstrate that the human and murine Site I could be efficiently competed by an idealized LXRα:RXR binding site, illustrating the specificity of the interaction. The results obtained demonstrate that the human and murine cyp7a gene promoter and subsequently their response to cholesterol. We surveyed the sequence of the human and murine cyp7a gene promoter and subsequently their response to cholesterol. We surveyed the sequence of the Site I in a small cohort of unrelated individuals from different ethnic backgrounds. This analysis revealed that the human Site I sequence was invariant (Supplemental Fig. 1), implying a similar general response of the human CYP7A1 gene to transcriptional control at this regulatory site.

In summary, we have described the creation of a mouse strain that uniquely expresses the human cyp7a enzyme in the liver. This model has permitted us to evaluate the response of the human CYP7A1 gene to dietary cholesterol in the intact animal, under the same conditions normally used to study murine Cyp7a1 gene expression but without interference from the functional consequences of the endogenous Cyp7a1 gene. The results obtained demonstrate that the human CYP7A1 gene is not stimulated by dietary cholesterol in vivo. Previously we showed that the murine Cyp7a1 gene promoter is induced by fats more robustly compared with the human CYP7A1 gene promoter in hepatoma cells (11). We have also found that the activity of the human CYP7A1 gene promoter is repressed by thyroid hormone (20), contrary to the response described for murine and rat Cyp7a1 gene promoters (21–24). These findings may be illustrating a fundamental difference in the way the human CYP7A1 and murine Cyp7a1 genes are normally regulated in vivo.

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