AN ALTERATION IN EXPRESSION OF THE CYTOCHROME P-450 2D GENE AFTER THE APPEARANCE OF JAUNDICE IN LEC RATS

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A marked decrease in drug metabolizing ability was observed after the appearance of jaundice, one of the clinical sings of the onset of the naturally occurring hepatitis in rats of the LEC (Long-Evans Cinnamon) strain, which is a mutant strain of rats displaying a hereditary tendency to develop hepatitis with severe jaundice. This study investigated the changes in the activity of the cytochrome P-450-dependent drug metabolism, the expression of cytochrome P-450 BTL, a member of the P-450 2D subfamily, and the molecular mechanisms affecting the expression of cytochrome P-450 BTL before and after the appearance of jaundice. The results were compared with those of a similar study using DA (Dark Agouti) rats which are an animal model for human debrisoquine/sparteine oxidation polymorphism and know to carry a mutation which results in the non-expression of the gene encoding P-450 2D1 (a member of the P-450 2D subfamily). The metabolic activities were determined by quantifying metabolites of bunitrolol and imipramine by HPLC. The expression of P-450 was confirmed by Western blotting analysis by using an antibody to P-450 BTL. The properties of DNA and the transcription to mRNA were investigated by extracting DNA and RNA from hepatocytes, PCR amplification with those nucleic acids, and finally by Northern blotting analysis and direct sequencing.

The activities and expression of P-450 BTL (antibody against which cross-reacts with P-450 2D1) were markedly decreased after the appearance of jaundice in LEC rats. The transcription to mRNA of the P-450 2D1 gene in liver of DA rats was not decreased. It has been reported by Matsunaga et al. (1989) that the absence of P-450 2D1 protein in liver of DA rats was caused by the lack of transcription. The present study using RT-PCR direct sequencing techniques indicated that at least a part of the P-450 2D1 gene exists in liver of DA rats. This suggests that the P-450 2D1 protein was not expressed in rats due either to a splicing error of 2D1 pre-mRNA, which is similar to the cause of human debrisoquine polymorphism characterized by the absence of the expression of the P-450 2D6 gene, or to the presence of a mutation somewhere downstream from the exon fragment used for direct sequencing in this study.