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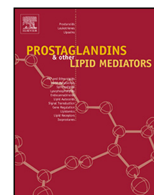

Original research article

Changes in gene expression of cytochrome P-450 in liver, kidney and aorta of cirrhotic rats

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ABSTRACT

Introduction: Liver cirrhosis is characterized by structural and hemodynamic changes that affect mainly the liver, the kidney and the vascular system. Cytochrome P-450 (CYP) is a variegated family of enzymes that, among many other activities, metabolize arachidonic acid to the vasoactive epoxyeicosatrienoic acids (EETs).
Aim: To investigate in an animal model of cirrhosis the m-RNA expression of CYPs in liver, kidney and aorta and to evaluate the effect of epoxygenase inhibition by *N*-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH).
Methods: In aorta, liver and kidney from 3 control, 3 cirrhotic and 6 cirrhotic rats treated with MS-PPOH, quantitative real-time PCR reactions were performed and the m-RNA expression of CYP2J3, CYP2J4, CYP2J10, CYP2C11, CYP2C12 and CYP2C23 was calculated.
Results: In cirrhotic rats, the gene expression of hepatic CYP2C11 and CYP2J10 was increased, of aortic CYP2J4 was increased, of aortic CYP2C12 was reduced and of renal CYP2C11 was increased. In cirrhotic rats, MS-PPOH reduced CYP2J10 hepatic and CYP2C11 renal gene expression to levels similar to the ones of control rats.
Conclusions: Changes in CYPs gene expression may contribute to the hemodynamic alterations typical of cirrhosis. The altered gene expression of CYPs can, in some cases, be reversed by epoxygenase inhibition.
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1. Introduction

It has long been recognized that cyclooxygenase and lipoxygenase enzymes metabolize arachidonic acid (AA) to 5-, 12- and 15-hydroxyeicosatetraenoic acid (HETE), prostaglandins, prostacyclin, thromboxane, and leukotrienes. However, more recently a third pathway for the metabolism of AA emerged: AA is also metabolized via cytochrome P-450 (CYP) enzymes to epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acids (DiHETEs) and HETEs [1].

More than 500 CYP genes have been identified and categorized by sequence homology [2]. These genes are subdivided into 78 families. Fourteen of these families (29 subfamilies) are expressed in mammalian tissue. Up to 50 different CYPs enzymes are expressed in a given species. Most of these enzymes are expressed in the liver and are involved in the metabolism of drugs. However, many CYPs enzymes are expressed in extrahepatic tissues and metabolize endogenous substrates like vitamins, steroids, and fatty acids, including arachidonic acid. Reports suggest that specific CYPs localize in each tissue and contribute to the regulation of homeostasis in tissue function. The CYPs in the CYP2C and 2J families are drug-metabolizing enzymes but they are also reported to produce EETs [3,4].

In liver cirrhosis, which is characterized by the replacement of normal hepatic tissue with fibrous tissue leading to progressive loss of liver function, portal pressure increases as a consequence of both increased vascular resistance and increased portal blood flow. Indeed, portal hypertension results from changes in the hepatic circulation that increase intrahepatic resistance and changes in the splanchnic circulation that lead to splanchnic vasodilatation, decreased response to vasoconstrictors and increased splanchnic blood flow. The increase in intrahepatic resistance is due not only to morphological changes occurring in this pathological condition, but also to contractile elements located at the sinusoidal level as well as at extrasinusoidal sites in the liver [5,6] which are able to constrict in a reversible and graded manner in response to several

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agonists [7]. A decreased hepatic NO availability is thought to be the major factor contributing to the increase in the intrahepatic vascular resistance in experimental cirrhosis, but also vasoconstrictive factors have a relevant effect on the hepatic vascular tone. Even if CYP-dependent AA metabolites EETs are known to cause vasodilatation in various arterial vascular beds and hyperpolarize vascular smooth muscle cells, 11,12-EET was shown to be a vasoconstrictor in the porto-sinusoidal circulation [8]. Different mechanisms have been suggested to explain splanchnic vasodilation in portal hypertension, which is likely to represent a multifactorial phenomenon involving neurogenic, humoral and local mechanisms [9]. Among other circulating vasodilators that have been involved in the pathogenesis of splanchnic vasodilation, EETs may serve as endothelial-derived hyperpolarizing factors (EDHF) [10]. Free plasma EETs are increased in cirrhotic patients compared to normal subjects [11]. Moreover, our group has demonstrated that in cirrhosis the increased vasodilation of mesenteric arterial circulation is due, at least in part, to an hyperreactivity to 11,12-EET through an increased expression of myoendothelial gap junctions [12]. Renal vasoconstriction leading to reduced renal function and renal failure is a common observation in cirrhosis. While in normal subjects 20-HETE and PGs are found at similar rates, in patients with cirrhosis urinary 20-HETE is several-fold higher than prostaglandins and thromboxane B2. This suggests that 20-HETE may be produced in higher amounts in the preglomerular microcirculation accounting for the functional decrease of flow and increase in sodium reabsorption [13].

The aim of this study was to evaluate in an animal model of cirrhosis the m-RNA expression of those CYPs whose presence had already been observed in rats (CYP2J3, CYP2J4, CYP2J10, CYP2C11, CYP2C12 and CYP2C23). The analysis was performed in those tissues more involved in the pathogenesis of portal hypertension and the hyperdynamic syndrome, i.e. the liver, the kidney and the vascular system (aorta). If in a tissue a difference in gene expression of a specific CYP was found between cirrhotic and control rats, the gene expression of that CYP was also measured in the same tissue obtained from cirrhotic animals treated with ***N***-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH), a potent and selective inhibitor of CYP-catalyzed AA epoxidation in vivo [14].

2. Materials and methods

2.1. Animals

The study was performed on 12 adult male Wistar rats (body weight, 275–300 g; Charles River Laboratories, Calco, Italy). The experiments were carried out in accordance with the legislation of Italian authorities (D.L. 27/01/1992 116), which complies with European Community guidelines (CEE Directive 86/609) for the care and use of experimental animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee. Cirrhosis was induced with the CCl4 inhalation method in 9 rats as described previously [15]. Phenobarbital (0.30 g/L) was added in the rats drinking water. Treatment was followed for 16 weeks, and animals were free of treatment for the last week before the experiment. Six of these rats underwent an intravenous injection of 1 ml of MS-PPOH in the tail vein (20 mg/kg/die) every 24 h for three days before the experiment. Under anesthesia with ketamine hydrochloride (100 mg/kg bw, i.m.), liver, kidneys and aorta were excised. Age-matched animals were used as controls.

2.2. mRNA expression of CYPs

RNA extraction, complementary DNA synthesis, quantitative real-time PCR (Q-PCR) reactions were performed as previously described [12] in aorta, liver and kidney of 3 control and 3 cirrhotic rats. Different cytocrome mRNA levels were measured by Q-PCR, using the SYBR® green method and fold changes were generated for each sample by calculating $2 - \Delta CT$ [16]. The amplification mix was prepared using Roche LightCycler FastStart DNA MasterPLUS SYBR Green I kit following manufacturer's instructions and real-time PCR was performed using LightCycler instrument (Roche). Oligonucleotide sequence of specific rattus primers used for Q-PCR were: sense, 5′-AAGCACAATCCGCAGTCTGA-3′, reverse 5′-GGCATCTGGCTCCTGTCTTT-3′ (for Cyp 2c11); sense, 5′-CAGAGATATATTGACCTTGCCCCA-3′, reverse 5′-CCTCTCCCACACATTTCCGT-3′ (for Cyp 2c12); sense 5′-TTGCCTCAGCCTCCTTTCAG-3′, reverse 5′-AACAGGCCCGTACTCTTTGG-3′ (for Cyp 2c23); sense, 5′-ATGCAAGAACGCATCACCAAT-3′, reverse 5′-AAGCAACCCACTAAGGGCAG-3′ (for Cyp 2j3); sense, 5′-CAGAACTCTGTTGCTCGCTG-3′, reverse 5′-AGTGTCATCAGGGCAAACCT-3′ (for Cyp 2j4); sense, 5′-CATTTGGACCGTGCTCCATC-3′, reverse 5′-AACTGGAACAAGTTGCCCAC-3′ (for Cyp 2j10); Gliceraldehyde-3-phosphate dehydrogenase (GAPDH) sense 5′-GGTGAAGGTCGGTGTGAACG-3′, reverse 5′-GTCACAAGAGAAGGCAGCCC-3′ was used as internal reference and co-amplified with target samples using identical Q-PCR conditions. Samples were run in triplicate and mRNA expression was generated for each sample. Specificity of the amplified PCR products was determined by melting curve analysis and confirmed by agarose gel electrophoresis.

The same experiments were performed in tissues obtained from 6 cirrhotic animals treated with MS-PPOH for those CYPs whose gene expression was found different between cirrhotic and control rats.

2.3. Data analysis

Data were analyzed by Student's *t* test for unpaired observations. The null hypothesis was rejected at $p < 0.05$.

3. Results

3.1. Hepatic, renal and aortic CYP2J3, CYP2J4, CYP2J10, CYP2C11, CYP2C12 and CYP2C23 gene expression in cirrhotic rats (Table 1)

In livers from both healthy and cirrhotic rats, CYP2C11 was by far the most expressed gene; moreover, its expression was significantly higher in the cirrhotic group ($p < 0.05$). On the contrary, the hepatic gene expression of CYP2C23 was the lowest observed, with no differences between cirrhotic and normal rats. In this organ, CYP2J10 gene expression was higher in cirrhotic rats as compared with controls ($p < 0.05$), while no differences in the expression of CYP2J3, CYP2J4 and CYP2C12 were observed.

In aorta, there was no marked difference in the gene expression between the different CYP enzymes as it was observed in the liver. In cirrhotic rats, CYP2J4 gene expression was increased ($p < 0.05$) while CYP2C12 was reduced ($p < 0.01$) as compared with controls. The levels of the other CYPs studied were similar in the two groups.

Similarly to what observed in liver, in kidneys from both groups, CYP2C11 was the most expressed gene and its expression was significantly increased in cirrhotic animals ($p < 0.01$). CYP2J3, CYP2J4, CYP2J10, CYP2C12 and CYP2C23 gene expression was similar in cirrhotic and control rats.

3.2. Effects of MS-PPOH administration on CYP gene expression in cirrhosis (Fig. 1)

In cirrhotic rats, MS-PPOH (Cayman Chemical, Ann Arbor, MI, USA) administration had no effects on aortic CYP2J4 and CYP2C12

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