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Original Article

Role of cyclooxygenase isoforms in encephalopathy of cirrhotic rats

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Abstract

Background: Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome secondary to acute or chronic liver failure. However, its pathophysiology remains obscure. Recently, we found that the inhibition of cyclooxygenase by indomethacin aggravated HE in rats with thioacetamide-induced acute hepatic failure, suggesting a pivotal role of cyclooxygenase in HE. This study was aimed at surveying the roles of cyclooxygenase isoforms responsible for prostaglandins synthesis, cyclooxygenase-1 (COX1) and COX2, in cirrhotic rats with HE.
Methods: Liver cirrhosis was induced (using formalin) in male Sprague−Dawley rats with bile duct ligation (FBDL). Sham-operated rats served as the surgical controls. The severity of HE was assessed by motor activity counts. Plasma 6-keto-prostaglandin-$F_{1\alpha}$ [6-keto-$PGF_{1\alpha}$; a relatively stable metabolite of prostacyclin (PGI_2)], alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALK-P), and total bilirubin were measured. Hepatic mRNA expressions of COX1 and COX2 were tested.
Results: The FBDL group showed lower motor activity counts than the sham group in total (1472 ± 156 vs. 2174 ± 262 counts/30 min, $p = 0.034$), ambulatory (824 ± 99 vs. 1443 ± 206 counts/30 min, $p = 0.014$), and vertical movement (431 ± 69 vs. 849 ± 145 counts/30 min, $p = 0.018$). The mRNA expression of hepatic COX2 was significantly higher in the FBDL group. Plasma ALK-P and bilirubin levels were negatively correlated with total movements, respectively (both $p < 0.05$). In addition, hepatic COX2 mRNA expression was positively correlated with AST, ALK-P, total bilirubin, and 6-keto-$PGF_{1\alpha}$ (all $p < 0.05$), but not correlated with total movements.
Conclusion: Hepatic COX2 expression and PGI_2 production are enhanced in cirrhotic rats, but the correlation with HE is not remarkable. Cyclooxygenase and PGI_2 may not play important roles in HE in the setting of chronic liver failure.

Keywords: bile duct ligation; cyclooxygenase; hepatic encephalopathy; liver cirrhosis; prostacyclin

1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric derangement associated with acute or chronic hepatic decompensation with or without portal-systemic shunts.[1,2] The disorder consists of a diversity of symptoms, varying from trivial mental instability or memory impairment to confusion, coma, and death. The initiation and maintenance of HE have been ascribed to several substances, such as ammonia,

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gamma-aminobutyric acid, benzodiazepines, aromatic amino acids, false neurotransmitters, endotoxin, and tumor necrosis factor-α.[1–3] However, the true nature mechanism remains not totally clarified.

Prostacyclin (PGI_2), a vasodilatory prostaglandin, has been postulated to play a role in the pathogenesis of hepatic failure with encephalopathy. PGI_2 participates in the mechanism of hyperdynamic circulation in portal hypertensive status. For instance, increased levels of 6-keto-prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a stable metabolite of PGI_2, have been noted in animals with portal hypertension and patients with liver cirrhosis.[4,5] The vasodilatory nature of PGI_2 may contribute to HE[6]: cerebral vasculature dilatation increases capillary surface and subsequently facilitates the diffusion of noxious gut-derived compounds, such as ammonia.[7] It has also been shown that PGI_2 alters the permeability of the blood–brain barrier.[8] Therefore, it is reasonable that PGI_2 may participate, at least partly, in the pathophysiology of HE.

The role of PGI_2 during hepatic injury seems to be quite controversial. Although raised blood prostaglandin levels have been noted in massive hepatic necrosis,[9] prostaglandins have been shown to ameliorate hepatic insult in terms of prolonged survival and histological improvements.[10] Our previous study showed a detrimental effect of indomethacin administration on HE in rats with thioacetamide (TAA)-induced fulminant hepatic failure, which is believed to be derived from nonselective prostaglandin synthesis inhibition.[11] Because indomethacin exerts a universal blockade of two prostaglandin synthesis isoenzymes, cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2), the roles of COX isoforms in HE, indeed, require further clarification.

As chronic liver parenchymal disease is a more prevalent form of hepatic failure and encephalopathy in clinical practice, we used bile duct-ligated cirrhotic rats to quantitatively survey the degree of HE with motor activities.[12–14] The plasma levels of 6-keto-$PGF_{1\alpha}$ and liver biochemistry parameters were also measured, and the hepatic COX isoform expressions were evaluated. Furthermore, the correlations between them were assessed.

2. Methods

2.1. Animal model

Male Sprague–Dawley rats weighing 240–270 g were used for this study. The rats were caged at 24°C, with a 12-hour light–dark cycle and free access to food and water until the time of the experiments. Rats with secondary biliary cirrhosis were induced by common bile duct ligation (BDL).[15] Under ketamine anesthesia (100 mg/kg, intramuscularly), the common bile duct was exposed through a midline abdominal incision, catheterized by a PE-10 catheter and doubly ligated with 3–0 silk. The first ligature was made below the junction of the hepatic ducts and the second above the entrance of the pancreatic duct. Ten percent formalin (~10 μL/100 g) was slowly injected into the biliary tree to prevent the subsequent dilatation of the ligated residual bile duct. Thus, this animal model is abbreviated as FBDL. The PE-10 catheter was then removed and the ligatures were tightened, followed by sectioning of the common bile duct between the ligatures. The incision was then closed, and the animal was allowed to recover. A high yield of secondary biliary cirrhosis was noted at least 4 weeks after the ligation.[15–17] To avoid coagulation defects, FBDL rats received weekly vitamin K injection (50 μg/kg, intramuscularly).[18] The studies were performed in overnight-fasted rats, 6 weeks after the operation. Two series of studies were performed. In the first series, after measurements of the motor activities, heparinized blood samples were obtained from the inferior vena cava for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALK-P), total bilirubin, and $PGF_{1\alpha}$ measurements ($n = 10$ for each group). In the second series, livers were dissected and removed for RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR; $n = 9$ for each group). The animal experiments were conducted according to *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985).

2.2. Measurement of motor activities

The severity of HE was quantified with motor activities. Motor activities in an open field were determined using the Opto-Varimex animal activity meter (Columbus Instruments, Inc., Columbus, OH, USA).[12,18] The Opto-Varimex activity sensors utilize high-intensity, modulated infrared light beams to detect animal motion. Animals were housed in transparent cages (17 × 17 × 8 inches), through which 30 infrared beams passed in the horizontal plane, 15 on each axis. This device differentiated nonambulatory movements (scratching, gnawing) from ambulation on the basis of consecutive interruption of the infrared monitoring beams. An additional row of infrared beams in the horizontal plane (15 on each axis) about 10 cm above the floor was used to count vertical movements. During the activity measurements, animals had no access to food or chow. All studies were performed under strictly standardized conditions in a dark room for 30 minutes. Counted numbers of total movements, ambulatory movements, and vertical movements were separately recorded to reflect the motor activities of the rats.

2.3. Determination of plasma 6-keto-$PGF_{1\alpha}$ levels

Heparinized blood was centrifuged at 1789*g* for 10 minutes, then the plasma was separated, added with indomethacin (10 μg/ml), and stored under −80°C. At the time of measurement, samples were thawed, and 6-keto-$PGF_{1\alpha}$ was determined with a commercially available enzyme immunoassay according to the protocol supplied by the manufacturer (R&D Systems, Minneapolis, MN, USA).

2.4. RNA extraction and RT-PCR

Total RNA was extracted from the vessel with an RNeasy Mini Kit according to the manufacturer's instructions (Qiagen

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