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Enhanced vasoconstrictor prostanoid production by sinusoidal endothelial cells increases portal perfusion pressure in cirrhotic rat livers☆

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***Background/Aims*: Cyclooxygenase-1 (COX-1) is overexpressed in sinusoidal endothelial cells (SEC) of cirrhotic rat livers, and through an enhanced production of vasoconstrictor prostanoids contributes to increase intrahepatic resistance. Our study was aimed at investigating the role of enhanced AA bioavailability modulating the hepatic vascular tone of cirrhotic livers and identifying which prostanoid is involved.**

***Methods*: SEC isolated from control and cirrhotic rat livers were incubated with AA, methoxamine or vehicle. TXA_2 was quantified. In addition, portal perfusion pressure (PP) response curves to AA were performed in rat livers pre-incubated with vehicle, SC-560 (COX-1 inhibitor), Furegrelate (inhibitor of TXA_2 synthesis) and SQ-29548 (PGH_2/TXA_2 receptor blocker). cPLA2 activity was determined in control and cirrhotic livers.**

***Results*: AA and methoxamine incubation promoted a significant increase in TXA_2 release by Cirrhotic-SEC, but not in Control-SEC. AA produced a dose-dependent increase in the PP, associated with increased TXA_2 release. These responses were significantly greater in cirrhotic livers. COX-1 inhibition and PGH_2/TXA_2 receptor blockade, but not TXA_2 synthase inhibition, markedly attenuated the PP response to AA of cirrhotic livers. Additionally, cirrhotic livers exhibited significantly increased cPLA2 activity.**

***Conclusions*: An enhanced production of vasoconstrictor prostanoids, probably PGH_2, by SEC contributes to increase vascular tone of cirrhotic livers.**

***Keywords*: Intrahepatic resistance; Phospholipase A2; Sinusoidal endothelial cells; Arachidonic acid; PGH_2**

1. Introduction

In cirrhotic livers, increased vascular resistance to portal blood flow is the initial factor leading to the development of portal hypertension [1]. This increase in resistance is, in part, due to reversible functional alterations including a deficient response of the hepatic vascular bed to vasodilators or an exaggerated response to vasoconstrictors [2–4]. Increased production of arachidonic acid (AA) derived vasoconstrictors via the cyclooxygenase-1 (COX-1) pathway has been shown to increase hepatic vascular resistance in cirrhosis [5–8].

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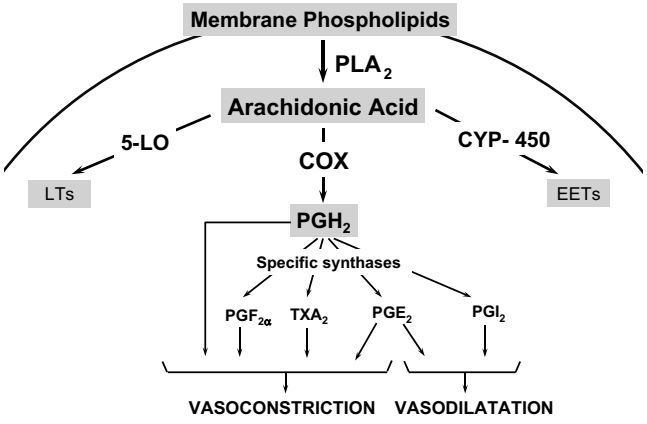

Fig. 1. Arachidonic acid (AA) metabolism pathway. AA is synthesized from membrane phospholipids by phospholipase A2 (PLA2). Once free AA could be transformed through three pathways forming different product families. 1 AA could be metabolised by 5-lipooxygenase (5-LO) to form leukotrienes (LTs), essential eicosanoid lipid mediators involved in inflammation and in vascular tone regulation. 2 By cyclooxygenase (COX) activity AA could be transformed to the common prostaglandin (PG) precursor and vasoconstrictor PGH2. PGH2 is rapidly metabolised by specific synthases to form the broad spectrum of prostaglandins family, including PGF2α, PGE2, PGI2 and thromboxane A2 (TXA2), all of them with potent vasoactive properties. 3 Finally AA could also be transformed to epoxyeicosatrienoic acids (EETs), metabolites with some vascular and tubular functions, by cytochrome P-450 (CYP-450).

Moreover, it has been suggested that these vasoconstrictor prostanoids would be mainly synthesized by cirrhotic sinusoidal endothelial cells (SEC) that overexpress COX-1 [7].

In several cell systems activation of cytosolic phospholipase A2 (PLA_2) by a G-protein coupled receptor-dependent mechanism promotes the release of AA from membrane phospholipids [9,10]. AA would then be metabolised to PGH_2 by COX. PGH_2 is the common precursor for prostaglandin (PGs) and thromboxane (TXs) by action of different specific PG and TX synthases [11] (Fig. 1). We hypothesize that, in the cirrhotic liver, an increase in AA bioavailability in an environment of increased COX-1 expression, such as in cirrhotic SEC, will contribute to enhanced production of vasoconstrictor prostanoids that would lead to increased hepatic vascular resistance.

Thus, the aim of the present study was to investigate the role of an enhanced AA bioavailability modulating the hepatic vascular tone of cirrhotic livers, regulating vasoconstrictor-prostanoid synthesis by SEC and identifying which is the main vasoconstrictor prostanoid involved.

2. Materials and methods

2.1. Induction of cirrhosis by CCl_4

Male Wistar rats weighing 175–200 g underwent inhalation exposure to CCl_4 and received phenobarbital in the drinking water as previously described [5]. Once the cirrhotic rats developed ascites, usually at week 16, administration of CCl_4 and phenobarbital was stopped and experiments were performed 1 week later. Control animals received only phenobarbital. The animals were kept in environmentally controlled animal facilities at the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). All experiments were performed according to the criteria of the Committee for the Care and Use of Laboratory Animals in the Hospital Clínic and IDIBAPS.

2.2. Liver sinusoidal endothelial cells isolation

Liver sinusoidal endothelial cells (SEC) were isolated from control ($n = 7$) and cirrhotic ($n = 7$) rat livers as described elsewhere [7,12] with minor modifications. Briefly, livers were perfused through the portal vein for 10 min at a flow rate of 20 mL/min at 37 °C with Hanks' Balanced Salt Solution (HBSS) without calcium and magnesium containing 12.5 mM Hepes (pH 7.4), 0.5 mM EGTA and 2% BSA. Then, 30 min at a flow rate of 5 mL/min at 37 °C with 0.015% collagenase A (for cirrhotic livers the concentration was increased by 25%), HBSS containing 12.5 mM Hepes (pH 7.4) and 4 mM $CaCl_2$. The resultant digested liver was excised and in vitro digestion was performed at 37 °C with 0.01% collagenase A, HBSS containing 12.5 mM Hepes (pH 7.4) and 4 mM $CaCl_2$ for 10 min. The cells were passed through nylon filters, collected in cold Krebs' buffer and centrifuged at 50*g* for 3 min. The supernatant was centrifuged at 800*g* for 10 min at 4 °C and the obtained pellet was resuspended in Dulbecco's PBS (DPBS) and centrifuged at 800*g* for 25 min through a two-step Percoll gradient (25–50%) at 4 °C. The interface of the gradient was enriched in Kupffer cells and SEC. This cell fraction was diluted in DPBS and centrifuged at 800*g* for 10 min. The cell pellet was resuspended in RPMI medium, seeded in plastic dishes and incubated for 30 min at 37 °C in humid atmosphere with 5% CO_2 in order to enhance SEC purity by selective adherence of Kupffer cells. Non-adherent cells were seeded in collagen-coated 24 multiwell dishes at a concentration of 10^6 cells/mL per well and incubated for 1 h (37 °C, 5% CO_2). After this time the medium was discarded and adhered cells were washed twice with DPBS and cultured for 12 h (37 °C, 5% CO_2) in RPMI-1640 as previously described [13]. Specific immunocytochemical marking using rat endothelial cell antigen RECA-1 showed that almost 93% of these cells were SEC with a viability of 95% (evaluated by trypan blue exclusion).

Studies were performed 12 h after SEC isolation to preserve its typical phenotype [14].

2.3. Isolated perfused liver system

A flow-controlled perfusion system was employed in this study, as described previously [15]. Livers were perfused with Krebs' buffer in a recirculation fashion with a total volume of 100 mL at a constant flow rate of 35 mL/min. An ultrasonic transit-time flow probe (model T201; Transonic Systems, Ithaca, NY) and a pressure transducer were placed on line, immediately ahead of the portal inlet cannula, to continuously monitor portal flow and perfusion pressure. Another pressure transducer was placed immediately after the thoracic vena cava outlet for measurement of outflow pressure. The flow probe and the two pressure transducers were connected to a PowerLab (4SP) linked to a computer using the Chart version 5.0.1 for Windows software (ADInstruments, Mountain View, LA). The average portal flow, inflow and outflow pressures were continuously sampled, recorded and afterwards analyzed.

The perfused rat liver preparation was allowed to stabilize for 30 min before the studied substances were added. The gross appearance of the liver, stable perfusion pressure and a stable buffer pH (7.4 ± 0.1) were measured during this period. If any viability criteria were not satisfied, the experiment was discarded.

2.4. SEC prostanoids production

Twelve hours monolayer cultures of SEC isolated from control and cirrhotic rat livers were pre-incubated for 15 min with vehicle or with the selective COX-1 inhibitor SC-560 (5 μM) then AA (40 μM) or its vehicle (ethanol 0.1%) was added. After 20 min 250 μL aliquots of