Simultaneous inhibition of TXA2 and PGI2 synthesis increases NO release in mesenteric resistance arteries from cirrhotic rats

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ABSTRACT

Our present study examines, in mesenteric resistance arteries, possible vasodilation alterations, and the role of NO and COX (cyclo-oxygenase) derivatives, in cirrhosis. The vasodilator response to acetylcholine was analysed in segments from control and cirrhotic rats. The effects of the non-specific COX inhibitor indomethacin, the specific COX-1 inhibitor SC-560 and the specific COX-2 inhibitor NS-398 were analysed in segments from both groups of rats. NO release was measured, and eNOS [endothelial NOS (NO synthase)], phospho-eNOS, iNOS (inducible NOS), COX-1 and COX-2 protein expression was also analysed. The effects of the TP receptor [TXA2 (thromboxane A2) receptor] antagonist SQ 29548, the TXA2 synthesis inhibitor furegrelate, the PGI2 (prostaglandin I2) synthesis inhibitor TCP (tranylcypromine) or TCP + furegrelate were only determined in segments from cirrhotic rats. The vasodilator response to acetylcholine was higher in segments from cirrhotic rats. Indomethacin, SC-560 and NS-398 did not modify the vasodilator response in control rats; however, indomethacin, NS-398 and TCP + furegrelate increased, whereas SC-560 did not modify and SQ 29548, furegrelate or TCP decreased, the vasodilator response to acetylcholine in cirrhotic rats. NO release was higher in cirrhotic rats. Furegrelate decreased, whereas TCP + furegrelate increased, the NO release in segments from cirrhotic rats. eNOS and COX-1 protein expression was not modified, whereas phospho-eNOS, iNOS and COX-2 protein expression was higher in cirrhotic rats. Therefore the increase in iNOS expression and eNOS activity may mediate increases in endothelial NO release. The COX-2 derivatives TXA2 and PGI2 may act simultaneously, producing a compensatory effect that reduces NO release and may limit the hyperdynamic circulation.

INTRODUCTION

Cirrhosis is associated with a hyperdynamic circulatory syndrome characterized by high cardiac output, and reduced systemic vascular resistance and arterial pressure. Vascular resistance in circulatory districts other than the splanchnic system can be normal or increased, depending on the degree of portal hypertension.
whereas splanchnic arterial vasodilation produces the hyperdynamic circulatory syndrome. The effects of endothelial factors, mainly NO and COX (cyclooxygenase) derivatives, such as prostacyclin [PGI2 (prostaglandin I2)] [1], are reported to be involved in the mechanism(s) leading to the splanchnic vasodilation.

Various endothelial factors are involved in the regulation of vascular resistance in normal and altered haemodynamic conditions, and we believe that these factors also probably influence resistance arteries in cirrhosis. NO is synthesized by a family of enzymes, which is divided into two classes: cNOS [constitutive NOS (NO synthase)] and iNOS (inducible NOS) [2,3]. Many investigations have demonstrated that enhanced NO release contributes to the hyperdynamic process occurring in cirrhosis [4,5], but exactly which NOS isoform(s) and to what extent is still controversial.

There are few studies analysing the role of COX derivatives in cirrhosis. Some of these studies have found PGI2 release to be altered, and this alteration appears to affect the hyperdynamic circulation observed in cirrhosis [5]. Although enhanced production of vasoconstrictive COX derivatives has been reported to contribute to the increased vascular tone of cirrhotic livers [6], hardly any reports have considered their possible role in the hyperdynamic splanchnic circulation. NOS and COX are involved in vascular tone regulation and changes in one are accompanied by alterations in the other [7].

Thus our present study determines, in mesenteric resistance arteries, the possible alterations in vasodilation and the role of NO and COX derivatives in cirrhosis.

**MATERIALS AND METHODS**

**Drugs and solutions**

The drugs used were l-NA (N\(^{G}\)-nitro-l-arginine) hydrochloride, acetylcholine chloride, DEA-NO (diethylenamine NONOate), SQ 29548, l-NAME (N\(^{G}\)-nitro-l-arginine methyl ester) hydrochloride, indomethacin, TCP (tranylcypromine), NS-398, SC-560, SQ 29548, furegrelate, tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid) and lucigenin. Stock solutions (10 mmol/l) of drugs were made in distilled water, except for noradrenaline, which was dissolved in an NaCl (0.9 %)/ascorbic acid (0.01 % w/v) solution, indomethacin, U46619, SQ 29548 and SC-560, which were solubilized in ethanol and administered from a prepared stock in such a way that the maximal ethanol concentration of the medium was less than 0.001 % (v/v), and NS-398, which was solubilized in DMSO. All stock solutions were kept at –20°C, and appropriate dilutions were made in KHS [Krebs-Henseleit solution (115 mmol/l NaCl, 2.5 mmol/l CaCl\(_2\), 4.6 mmol/l KCl, 1.2 mmol/l KH\(_2\)PO\(_4\), 1.2 mmol/l MgSO\(_4\)·7H\(_2\)O, 25 mmol/l NaHCO\(_3\), 11.1 mmol/l glucose and 0.03 mmol/l EDTA) on the day of the experiment.

**Animals**

Male Sprague-Dawley rats (6 months old) were used. These were divided into two groups: control and cirrhotic rats. All animals were housed in the Animal Facility of the Universidad Autónoma de Madrid (Registration number EX-021U) in accordance with directives 609/86 CEE and R.D. 233/88 of the Ministerio de Agricultura, Pesca y Alimentación de Spain.

Cirrhosis was induced when rats were 4 months old by intragastric administration of CCl\(_4\) (carbon tetrachloride; 0.2 ml/100 g of body weight, twice a week; diluted 1:4 in olive oil) along with phenobarbital in the drinking water (0.35 g/l) over 9 weeks, as described previously [8,9].

Systemic BP (blood pressure) was indirectly measured in conscious animals by the tail-cuff method [10] using a LE5000 Digital Pressure Meter (Letica). Rats were killed by CO\(_2\) inhalation, and the mesenteric vascular bed was removed and placed in ice-cold (4°C) KHS.

**Mesenteric venous vasculopathy and portosystemic collateral circulation study**

The existence of portal hyperpressure in cirrhotic rats was confirmed by the development of mesenteric venous vasculopathy and portosystemic collateral circulation. First, a midline abdominal incision with a large bilateral subcostal extension was performed. Mesenteric venous vasculopathy, a characteristic feature of splanchnic venous congestion, was observed as dilation and tortuosity of the superior mesenteric vein branches [11].

The portosystemic collateral circulation was studied by macroscopic examination of the areas in which the collateral venous circulation had developed (splenorenal, gastroesophageal, colorectal and liver hilum), carefully identifying the development of collateral veins [12].

**Lipid and transaminase levels**

Blood samples were collected by cardiac puncture before the animals were killed. After centrifugation for 15 min at 1500 g, the serum was transferred to polypropylene tubes and then frozen at –80°C. The serum levels of liver transaminases GOT (glutamic oxaloacetic transaminase) and GPT (glutamate-pyruvic transaminase), cholesterol, triacylglycerols (triglycerides), HDL (high-density lipoprotein) and LDL (low-density lipoprotein) were measured by spectrophotometric colorimetric techniques, according to the manufacturer’s instructions (SpinReact).

**Vascular reactivity study**

For vascular reactivity experiments, the third-order branch of the mesenteric arcade (318±6.3 and 310±9.7 μm internal diameter in control and cirrhotic
rats respectively; $P > 0.05$) was dissected from the mesenteric bed, cleaned of connective tissue and cut into segments of approx. 2 mm in length. Two tungsten wires (40 μm in diameter) were introduced through the lumen of the segments and mounted in a small vessel chamber myograph (Danish Myo Technology) to measure isometric tension, according to the method described by Mulvany and Halpern [13]. After an equilibration period of 30 min in oxygenated (95%O2/5% CO2) KHS at 37 °C and pH 7.4, segments were stretched to their optimal lumen diameter for active tension development. This was determined based on the internal circumference/wall tension ratio of the segments by setting their internal circumference, $L_\text{o}$, to 90% of what the vessels would have been if they were exposed to a passive tension that was equivalent to the tension produced by a transmural pressure of 100 mmHg [13].

**Experimental protocols**

After the equilibration period of 30 min, arteries were exposed twice to 120 mmol/l KCl to check their functional integrity. Afterwards, concentration–response curves to acetylcholine (1 nmol/l–1 μmol/l) were made in arterial segments previously contracted with noradrenaline at a concentration that produced close to 50% of the contraction induced by KCl (120 mmol/l). This curve was constructed in the absence and presence of the NOS inhibitor l-NAME (100 μmol/l).

To determine the role of COX derivatives in the vasodilator response to acetylcholine, the non-specific COX inhibitor indomethacin (10 μmol/l), the constitutive COX-1 inhibitor SC-560 (50 nmol/l) or the inducible COX-2 inhibitor NS-398 (10 μmol/l) were added to the organ chamber 30 min before the concentration–response curve to acetylcholine was constructed. In another set of experiments, segments were incubated with the TP receptor (TXA2 [TX (thromboxane) A2 receptor] antagonist SQ 29548 (1 μmol/l), the PGI2 synthesis inhibitor TCP (10 μmol/l), the TXA2 synthase inhibitor furegrelate (1 μmol/l) or a combination of both TCP and furegrelate to assess the effect of these drugs on NO release, only in segments from cirrhotic rats. The amount of NO released is expressed as arbitrary units/mg of tissue.

**Detection of superoxide anions**

In order to study the effect of liver cirrhosis on superoxide anion release, the second, third and fourth branches of the mesenteric artery from either control or cirrhotic rats were equilibrated for 30 min in Hepes buffer at 37 °C, transferred to test tubes that contained 1 ml of Hepes buffer (pH 7.4) containing lucigenin (250 μmol/l) and then kept at 37 °C. The luminometer was set to report arbitrary units of emitted light; repeated measurements were collected over 5 min at 10 s intervals and averaged. Tiron (10 mmol/l), a cell permeant and non-enzymatic scavenger of the superoxide anion, was added to quench superoxide-anion–dependent chemiluminescence. In addition, blank measurement samples were collected from medium without resistance mesenteric segments in order to calculate and subtract the background emission. Some assays were performed in the presence of l-NAME (0.1 mmol/l), TCP (10 μmol/l), furegrelate (1 μmol/l) or TCP + furegrelate to assess the effect of these drugs on NO release, but only in segments from cirrhotic rats. The amount of NO released is expressed as arbitrary units/mg of tissue.

**Prostanoids and CRP (C-reactive protein) production**

To measure the release of CRP, and the stable metabolites of TXA2 and PGI2, TXB2 and 6-keto-PGF1α respectively, a rat CRP ELISA kit (BD Biosciences), and TXB2 and 6-keto-PGF1α EIA kits (Cayman Chemical) were used. The second, third and fourth branches of mesenteric resistance arteries from control and cirrhotic rats were pre-incubated for 45 min in 200 μl of KHS at 37 °C and
continuously gassed with a 95% O₂/5% CO₂ mixture (stabilization period). Afterwards, three washout periods of 7 min each in a bath of 200 μl of KHS were performed before incubation with acetylcholine (0.1 nmol/l–0.1 μmol/l). The medium was only collected at the end of the concentration–response curve to acetylcholine. Some samples were collected in the presence of 1 μmol/l furegrelate in order to determine possible variations produced by this inhibitor. The different assays were performed following the manufacturer’s instructions. Results are expressed as ng of CRP · ml⁻¹ · mg⁻¹ of tissue, or pg of prostanoid · ml⁻¹ · mg⁻¹ of wet tissue.

Western blot analysis
For Western blot analysis, the second, third and fourth branches of the mesenteric artery from both groups of rats were homogenized in a boiling buffer [1 mmol/l sodium vanadate, 1 % (w/v) SDS and 100 mmol/l Tris/HCl (pH 7.4)]. Homogenates containing 30 μg of protein were separated electrophoretically by SDS/PAGE (7.5% polyacrylamide gel), and then transferred on to PVDF membranes (Immun-Blot; Bio-Rad Laboratories). The membranes were incubated with the appropriate secondary HRP (horseradish peroxidase)-conjugated IgG antibody (1:500 dilution; Cayman Chemical). After washing, the immunocomplexes were detected by its phosphorylation at Ser1177, 1:500 dilution; polyclonal antibody against phospho-eNOS (assessed 1:2500 dilution; Transduction Laboratories), a rabbit polyclonal antibody against eNOS (endothelial NOS; 1:5000 dilution; Transduction Laboratories), a mouse monoclonal antibody against COX-1 (1:500 dilution; Abcam Laboratories), a mouse monoclonal antibody against COX-2 (1:500 dilution; Cayman Chemical) or a rabbit polyclonal antibody against COX-2 (1:500 dilution; Cayman Chemical). After washing, the membrane was incubated with the appropriate secondary HRP (horseradish peroxidase)-conjugated IgG antibody (Amershams Biosciences). The membrane was washed thoroughly and the immunocomplexes were detected using an enhanced HRP/luminol chemiluminescence system (ECL Plus; Amershams Biosciences) and subjected to autoradiography (Hyperfilm ECL; Amershams Biosciences). Signals on the immunoblot were quantified using a computer program (NIH Image version 1.56). The same membrane was used to determine α-actin expression, and the content of the latter was used to correct for protein expression in each sample by means of a monoclonal antibody against α-actin (1:2000 dilution; Sigma).

Statistical analysis
Results are means ± S.E.M. for the number of rats indicated. A two-way ANOVA was used to compare the curve obtained in the presence of the different substances with the control curve. For the NO, prostanoids and CRP release experiments, an unpaired Student’s t test was used. A P value <0.05 was considered significant.

RESULTS
Evolution of cirrhosis
Lever cirrhosis did not modify systemic BP, but body weight was lower in cirrhotic animals. All cirrhotic animals had spleen hypertrophy and hepatomegaly (Table 1). The liver histology of cirrhotic animals showed a finely granulated surface and the histological features of cirrhosis (results not shown).

In the cirrhotic group, rats had portosystemic collateral circulation (pararectal, parasaophaeleal, splenorenal and portalhepatic collateral vessels).

Table 1 Effect of liver cirrhosis on body weight, systemic BP, spleen weight and liver weight
Results are means ± S.E.M. (n = 10 animals in each group). *P < 0.05 compared with control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cirrhotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>525.5 ± 6.4</td>
<td>487.9 ± 6.9*</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>149.6 ± 4.1</td>
<td>144.4 ± 3.4</td>
</tr>
<tr>
<td>Spleen weight/body weight (%)</td>
<td>0.1 ± 0.008</td>
<td>0.2 ± 0.009*</td>
</tr>
<tr>
<td>Liver weight/body weight (%)</td>
<td>3.0 ± 0.1</td>
<td>4.1 ± 0.1*</td>
</tr>
</tbody>
</table>

Table 2 Effect of liver cirrhosis on GPT, GOT, LDL, HDL, triacylglycerols and cholesterol levels
Results are means ± S.E.M. (n = 10 animals in each group). *P < 0.05 compared with control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cirrhotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT (units/l)</td>
<td>53.20 ± 4.9</td>
<td>100.30 ± 11.8*</td>
</tr>
<tr>
<td>GOT (units/l)</td>
<td>92.50 ± 6.6</td>
<td>135.20 ± 14.6*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>5.25 ± 0.4</td>
<td>10.80 ± 0.8*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>31.73 ± 4.2</td>
<td>20.94 ± 1.2*</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>50.40 ± 5.2</td>
<td>34.20 ± 4.6*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>92.00 ± 7.1</td>
<td>86.80 ± 9.9</td>
</tr>
</tbody>
</table>

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Role of prostaglandin in cirrhotic resistance vessels

Vascular reactivity experiments
To provide maximal activation for each preparation, segments were contracted with 120 mmol/l KCl; responses obtained were similar in vessels from control and cirrhotic rats (2.81 ± 0.11 compared with 2.88 ± 0.15 mN/mm respectively; \( P > 0.05 \)). Acetylcholine caused a cumulative concentration-dependent relaxation in noradrenaline-pre-contracted rat mesenteric resistance arteries, which was greater in segments from cirrhotic rats (Figure 1). In vessels from both groups, pre-incubation with L-NAME significantly reduced this response (Figure 1). In the presence of L-NAME, relaxation in response to acetylcholine was similar in vessels from both control and cirrhotic rats (Figure 1).

Pre-treatment with indomethacin, SC-560 or NS-398 did not modify vasodilator responses in mesenteric segments from control rats (Figure 2A). However, both indomethacin and NS-398 increased the vasodilator response to acetylcholine in segments from cirrhotic rats, whereas SC-560 did not modify the response (Figure 2B).

Pre-incubation with SQ 29548, furegrelate or TCP decreased the vasodilator response in segments from cirrhotic rats (Figure 3). Simultaneous pre-incubation with both TCP and furegrelate together increased the concentration–response vasodilation in response to acetylcholine in segments from cirrhotic rats (Figure 3). In arteries from control rats, relaxation in response to acetylcholine remained unmodified in the presence of SQ 29548, furegrelate, TCP or TCP + furegrelate (results not shown).

The vasodilator response to DEA-NO and the vasoconstrictor response to U46619 were similar in both experimental groups (results not shown).

NO release
Both basal and acetylcholine-induced NO release were higher in segments from cirrhotic rats (Figure 4). Pre-incubation with TCP did not modify either basal or acetylcholine-stimulated NO release in segments from cirrhotic rats (Figure 4). Furogrelate decreased both basal and acetylcholine-induced NO release in segments from cirrhotic rats, whereas a combination of TCP + furegrelate increased both basal

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Figure 1  Vasodilator response to acetylcholine in mesenteric resistance arteries from control and cirrhotic rats in the absence and presence of 100 μmol/l L-NAME
Results are means ± S.E.M., with the number of animals used indicated in parentheses.

Figure 2  Effect of pre-incubation with 10 μmol/l indomethacin, 10 μmol/l NS-398 or 50 nmol/l SC-560 on the vasodilator response to acetylcholine in mesenteric resistance arteries from control (A) and cirrhotic (B) rats
Results are means ± S.E.M., with the number of animals used indicated in parentheses.
and acetylcholine-induced NO release in segments from cirrhotic rats (Figure 4).

**Superoxide anion release**

Liver cirrhosis increased superoxide anion release by mesenteric resistance arteries (10.50 ± 1.85 units · min⁻¹ · mg⁻¹ of tissue in the control group compared with 29.25 ± 3.12 units · min⁻¹ · mg⁻¹ of tissue in the cirrhotic group; \( P < 0.05 \); \( n = 5 \) in each group).

**CRP release**

Basal CRP release was increased by liver cirrhosis (4.083 ± 0.78 ng · ml⁻¹ · mg⁻¹ of tissue in the control group compared with 23.20 ± 6.65 ng · ml⁻¹ · mg⁻¹ of tissue in the cirrhotic group; \( P < 0.05 \); \( n = 5 \) in each group). Acetylcholine increased CRP release in both groups, but the release was higher in segments from cirrhotic rats (14.93 ± 3.48 ng · ml⁻¹ · mg⁻¹ of tissue in the control group compared with 47.67 ± 12.27 ng · ml⁻¹ · mg⁻¹ of tissue in the cirrhotic group; \( P < 0.05 \); \( n = 5 \) in each group).

**Prostanoids release**

Liver cirrhosis did not modify basal TXB₂ release, but decreased acetylcholine-induced TXB₂ release in mesenteric resistance arteries (Figure 5A). Both basal and acetylcholine-induced 6-keto PGF₁α levels were decreased by liver cirrhosis (Figure 5B). Pre-incubation with furegrelate did not modify 6-keto PGF₁α levels in mesenteric segments from cirrhotic rats (Figure 5B).
Role of prostaglandin in cirrhotic resistance vessels

Liver cirrhosis did not modify eNOS or COX-1 expression, but phospho-eNOS, iNOS and COX-2 expression were all increased (Figure 6).

DISCUSSION

In the present study, CCl₄ treatment was maintained for 9 weeks, and all CCl₄-treated rats used in these experiments macroscopically had macro-/micronodular cirrhosis of the liver, hepatomegaly and spleen hypertrophy. No rat had ascites, which has only been described after 12–20 weeks of CCl₄ administration in these animals [15]. Transaminase levels (GOT and GPT) were increased after CCl₄ treatment. Additionally, in our experimental model, HDL and triacylglycerol levels were decreased, and LDL levels were increased, but total cholesterol was not modified in serum samples. These findings are in agreement with previous studies performed in portal hypertensive rats [16]. All of these alterations confirm that our experimental model develops liver cirrhosis.

In the present study, we have observed that, as reported in conductance mesenteric arteries [17], cirrhosis increases vasodilation induced by acetylcholine in mesenteric resistance arteries. This effect is associated with an increase in NO release while the vasodilator response to DEA-NO remained unmodified, confirming the major role played by NO release in the development of the hyperdynamic circulation. This effect was accompanied by an increase in iNOS expression, as described previously in the aorta and superior mesenteric arteries [18]. Additionally, in spite of the lack of modification of eNOS expression, we observed that eNOS phosphorylation at Ser₁₁₁⁷ seven increased in cirrhotic rats (Figure 6), indicating increased eNOS activation produced by liver cirrhosis. These observations do not agree with previous reports describing both a decrease and a lack of modification in superior mesenteric artery eNOS expression [17,18], and a decrease in eNOS activity in the aorta and superior mesenteric artery has also been described [18]. This discrepancy may be due to a difference in the vascular bed analysed.

Vascular alterations could be a consequence of oxidative stress [19,20], so we analysed the release of superoxide anions in our experimental conditions. The results obtained showed, as has been observed in cirrhotic livers [21], an increase in superoxide anion levels in mesenteric segments from cirrhotic rats.

One of the most commonly used inflammatory markers is CRP, which can predict a risk of coronary events [22,23]. This marker has been reported to increase in short-time portal hypertension with and without cirrhosis [24]. The increased CRP levels observed in our experimental conditions confirm an increase in inflammatory processes due to liver cirrhosis.

As a number of studies have already focused on the role of COX-derived PGI₂ and TXA₂ in the inflammatory process observed in cirrhosis [5,25], the next objective was to analyse the possible participation of COX derivatives in the vasodilator response induced by acetylcholine in mesenteric resistance arteries from cirrhotic rats. Indomethacin, NS-398 or SC-560 did not have any effect on the vasodilator response in control segments, indicating a lack of participation of COX derivatives in this experimental condition, as reported previously [26], whereas vasodilation increases in cirrhotic segments pre-incubated with indomethacin or NS-398, but not with SC-560. These results therefore indicate that COX-2-derived arachidonic acid metabolites modulate the endothelium-dependent relaxation in mesenteric resistance arteries from cirrhotic rats, but not from healthy ones. Supporting these results is the fact that
COX-2 is overexpressed in segments from cirrhotic rats, whereas COX-1 expression is not modified. Taken together, these results support the existence of an inflammatory process underlining the development of liver cirrhosis [22].

Our next objective was to analyse which COX-2 derivative was implicated in this effect. In our experimental model we have observed that PGI2 levels increased after the establishment of liver cirrhosis, as has been reported for circulating PGI2 levels in cirrhotic patients and portal hypertensive rats [27,28]. Numerous studies have demonstrated that PGI2 promotes vasodilation in various vascular beds by stimulating IP receptors (PGI2 receptors) and thereby increasing the intracellular cAMP concentration. In mesenteric arteries from cirrhotic rats, the relaxation in response to acetylcholine was diminished by incubation with the PGI2 synthesis inhibitor TCP, indicating that PGI2 has a functional role in the vasodilator response to acetylcholine and would consequently participate in the increased vasodilation associated with cirrhosis. NO release was not modified by TCP treatment, thus ruling out possible NO participation in this response. This result is in contrast with earlier reports in a similar experimental model that indicated a minor role for PGI2 in the development of the hyperdynamic circulation [29].

Changes in TXA2 release have also been suspected in cirrhosis. In the present study, we have observed that liver cirrhosis causes a decrease in TXA2 levels. Additionally, the TXA2 analogue U46619 induces vasoconstriction through TP receptor activation. Incubation with the TP receptor antagonist SQ 29548 increases the vasodilator response to acetylcholine in arteries from cirrhotic rats, suggesting that TXA2 could participate as a vasoconstrictor in the response to this endothelium-dependent vasodilator agent. However, inhibition of TXA2 synthesis by furegrelate induces a decrease in the vasodilator response to acetylcholine, suggesting that inhibition of TXA2 synthesis induces some other mechanism that would affect the vasomotor response to acetylcholine. Interactions between COX derivatives are known to be complex; the synthesis of one prostanoid is usually accompanied by changes in the synthesis of the other. This imbalance can be shifted towards hypoor hyper-compensatory responses [30]. Therefore we analysed the release of the PGI2 metabolite 6-keto PGF1α after pre-incubation with furegrelate in segments from cirrhotic rats. Our results show that 6-keto PGF1α release was not modified by pre-incubation with furegrelate, ruling out the possibility that furegrelate induced a new equilibrium in prostanoid synthesis in segments from cirrhotic animals. Increasing evidence indicates that there is considerable ‘cross talk’ between the NO and COX derivative biosynthetic pathways, resulting in an active modulation in several situations [7,31]. These findings lead us to hypothesize a possible modulation of NO synthesis by TXA2 in arteries from cirrhotic rats. Our results indicate that NO release was diminished in the presence of the TXA2 synthesis inhibitor furegrelate, thus explaining the decrease in the vasodilator response to acetylcholine after TXA2 synthesis inhibition.

These results appear to show that both COX-2 derivatives, PGI2 directly and TXA2 indirectly by increasing NO formation, play a role in maintaining the hyperdynamic circulation observed in resistance mesenteric arteries from cirrhotic rats; the results also suggest that inhibition of both TXA2 and PGI2 would have an additive effect. Thus our next objective was to analyse the effect of the simultaneous inhibition of both TXA2 and PGI2 synthesis on the response to acetylcholine. Surprisingly, we observed that the relaxation induced by acetylcholine was significantly increased in the presence of TCP + furegrelate.

In line with this possible COX–NO interaction we hypothesized that NO release would increase if TXA2 and PGI2 synthesis were inhibited simultaneously. Our hypothesis was confirmed, as pre-incubation with furegrelate combined with TCP increased NO release, and this would explain the increase in the vasodilator response to acetylcholine.

Additionally, the fact that simultaneous TXA2 and PGI2 synthesis inhibition produces exactly the same effect on the vasodilator response to acetylcholine as COX-2 inhibition excludes the participation of the other COX-2 derivatives in this effect. To our knowledge, this is the first time that the joint action of two COX-2 derivatives, PGI2 and TXA2, has been described to induce a different effect on NO release than either of them alone. Additionally, these results indicate that activation of both NOS and COX-2 plays a relevant role in the modulation of the vasodilator response to acetylcholine in cirrhosis, and that they lead to opposite effects: while NO appears to participate in hyperdynamic circulation development, the COX-2 derivatives TXA2 and PGI2 act together, appearing to produce a compensatory mechanism inhibiting NO.

The use of COX inhibitors, such as indomethacin, has been suggested to prevent liver fibrosis, as well as to attenuate the increase in portal hypertension produced in liver cirrhosis [32,33]. However, our results show that, as well as these beneficial effects, the use of COX inhibitors could worsen the hyperdynamic circulation associated with liver cirrhosis.

In conclusion, the present study shows that, in mesenteric resistance arteries from cirrhotic rats, increased iNOS expression and eNOS activity mediate increases in endothelial NO release. The COX-2 derivatives TXA2 and PGI2 act simultaneously, producing a compensatory effect that reduces NO release and could limit the hyperdynamic circulation.
AUTHOR CONTRIBUTION

Fabiano Xavier and Javier Blanco-Rivero performed the experiments, analysed the data, discussed the results and wrote the manuscript; Esther Sastre performed some of the experiments and analysed the data; Lina Badimón was involved in discussing the results; and Gloria Balfagón was involved in discussing the results and writing the manuscript.

ACKNOWLEDGEMENTS

We thank Mr Pablo Catalina for his technical assistance.

FUNDING

This work was supported by the Comisión Interministerial de Ciencia y Tecnología de España [grant numbers SAF2009-10374 and DEP2006-56187-C04-04].

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