Effects of the combined administration of propranolol plus sorafenib on portal hypertension in cirrhotic rats

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Am J Physiol Gastrointest Liver Physiol 302: G1191–G1198, 2012. First published March 8, 2012; doi:10.1152/ajpgi.00252.2011.—Low doses of sorafenib have been shown to decrease portal pressure (PP), portal-systemic shunts, and liver fibrosis in cirrhotic rats. Nonselective beta blockers (NSBB) are the only drugs recommended for the treatment of portal hypertension. The aim of our study was to explore whether the combination of propranolol and sorafenib might show an additive effect reducing PP in cirrhotic rats. Groups of common bile duct-ligated cirrhotic rats (CBDL) and sham-operated control rats were treated by gavage with vehicle, propranolol (30 mg·kg⁻¹·day⁻¹), sorafenib (1 mg·kg⁻¹·day⁻¹), or propranolol+sorafenib. Treatment began 2 wk after the CBDL or sham operation. Hemodynamic evaluation was performed after 2 wk of treatment. In cirrhotic rats, propranolol and sorafenib produced a significant (P < 0.001) and similar reduction in PP (∼19 and ∼15%, respectively). This was achieved through different mechanisms: whereas propranolol decreased PP by reducing portal blood flow (∼35%; P = 0.03), sorafenib decreased PP without decreasing portal flow indicating decreased hepatic resistance. After propranolol+sorafenib, the fall in PP was significantly greater (∼30%; P < 0.001) than with either drug alone, demonstrating an additive effect. However, the reduction in portal flow (∼39%) under combined therapy was not significantly greater than after propranolol alone. Sorafenib, alone or in combination with propranolol, produced significant reduction in portal-systemic shunting (∼25 and ∼33%, respectively), splanchnic vascularization (∼37 and ∼41%, respectively), liver fibrosis (∼38%), and hepatic neovascularization (∼42 and ∼51%, respectively). These effects were not observed after propranolol alone. In conclusion, the combination of propranolol+sorafenib causes a greater reduction in PP than either drug alone and decreases markedly the extent of portal-systemic shunting, splanchnic and hepatic neovascularization, and liver fibrosis, suggesting that this drug combination is a potentially useful strategy in the treatment of portal hypertension.

Portal hypertension is a common clinical syndrome that accounts for most of complications of cirrhosis of the liver, which is characterized by an increased portal pressure and by the formation of portal-systemic collaterals including the gastroesophageal varices (5).

Portal hypertension in cirrhosis is initiated by an increased hepatic resistance to portal blood flow caused by the distortion of liver vascular architecture and by an increased hepatic vascular tone due to intrahepatic vasoconstriction secondary to an imbalance between decreased endogenous dilators [mainly nitric oxide (NO)] and increased vasoconstrictor stimuli (5).

Traditionally, the increased splanchnic blood flow has been attributed to an overproduction of endogenous vasodilators and a decreased vascular reactivity to vasoconstrictors (5, 21), whereas the formation of collaterals has been considered a mechanical consequence of the increased portal pressure that results in the opening and dilatation of preexisting vascular channels at sites of anatomical communications between the portal and systemic circulations (5, 21). However, recent studies challenge these concepts by demonstrating that angiogenesis, the formation of new blood vessels from preexisting vasculature, plays a major role in the development and maintenance of splanchnic hyperemia and portosystemic collateralization (7, 11, 13, 15). Importantly, growing evidence suggests that pathological angiogenesis is also involved in the establishment of the abnormal angioarchitecture distinctive of the cirrhotic liver and is also related to fibrogenesis (4, 12, 27, 32).

Indeed, increased splanchnic neovascularization, regulated through the coordinated action of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), has been shown to have a crucial role in developing and maintaining portal hypertension, hyperdynamic splanchnic circulation, and portosystemic collateralization (11–13).

At present, nonselective beta blockers (NSBB) (e.g., propranolol and nadolol) are the only drugs recommended for primary and secondary prophylaxis against a variceal bleeding (10). These drugs decrease the splanchnic blood inflow by decreasing the cardiac output and also by blocking the adrenergic dilatory tone in mesenteric arterioles, which results in unopposed α-adrenergic-mediated vasoconstriction and, therefore, in a further decrease in portal inflow. NSBB, by decreasing splanchnic blood flow, also reduce shear stress, NO production, and NO synthase (eNOS) activity (30, 33), which could influence splanchnic neovascularization (1).

Sorafenib is a potent multikinase inhibitor that has been approved for treatment of hepatocellular carcinoma (26); this drug has powerful antiangiogenic effect since it blocks the VEGF receptor 2 (VEGFR-2) and PDGF receptor β (PDGFR-β) activation and RAF serine/threonine kinases cascade (36). According to a recent study published by our group (29), the administration of sorafenib to cirrhotic rats provides several beneficial effects. This treatment resulted in 80% decrease in splanchnic neovascularization, 18% decrease in the extent in portosystemic collateral vessels, and 25% reduction in portal

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pressure. Treatment with sorafenib also resulted in improvement in intrahepatic fibrosis, inflammation, and pathological angiogenesis.

The aim of our study was to explore whether the combination of propranolol, a NSBB that lowers portal pressure by decreasing portal blood inflow, and a low dose of sorafenib, a multi kinase inhibitor with antiangiogenic effects, might show an additive effect reducing portal pressure and portosystemic collateral formation in cirrhotic rats.

**MATERIALS AND METHODS**

**Materials.** Sorafenib was purchased from Bayer Pharmaceuticals (West Haven, CT). Propranolol was obtained from Sigma-Aldrich (Lyon, France). 51Cr-labeled microspheres were obtained from Perkin-Elmer (Boston, MA). Anti-von Willebrand factor (vWF) antibody, secondary antibody (Envision), and dianinobenzidine for immunohistochemistry were obtained from Dako (Carpinteria, CA). All other reagents were obtained from Sigma-Aldrich.

**Animals.** In this study male Sprague-Dawley rats (275–300 g) were used. All procedures were approved by the Committee for Care and Use of Laboratory Animal of the University of Barcelona and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1996). Secondary biliary cirrhosis was induced by common bile duct ligation (CBDL) (2). Briefly, the intervention consists of the isolation of the common bile duct followed by a double ligature with 5-0 silk thread. The first ligature is made below the junction of the hepatic ducts and the second one is made above the pancreatic ducts. Finally, the portion between the two ligatures is resected. This model develops biliary fibrosis-cirrhosis in 4 wk. Two weeks after the ligation, rats already present mild portal hypertension, and at 4 wk, severe portal hypertension, hyperdynamic circulation, portal-systemic shunts and ascites (2). Two days after surgery, the presence of bilirubin in the urine turned it a dark brown color, indicating successful ligation. For every group of treated rats, a group of sham-operated (SHAM) control rats was used. In SHAM animals the common bile duct was similarly manipulated but not ligated.

**Treatments.** The CBDL or SHAM rats were subdivided into four groups to be treated with 1) vehicle (0.9% sodium chloride) (CBDL, n = 7; SHAM, n = 5); 2) propranolol (30 mg·kg⁻¹·day⁻¹) (CBDL, n = 8; SHAM=6); 3) sorafenib (1 mg·kg⁻¹·day⁻¹) (CBDL, n = 8; SHAM, n = 6); 4) propranolol+sorafenib (CBDL, n = 8; SHAM, n = 5). All the treatments were administered orally by gavage, once a day. Treatments began 2 wk after CBDL or SHAM operation, when cirrhosis and portal hypertension were partially established, and were continued for 2 wk, after which hemodynamic studies were performed. Doses and schedule of administration of sorafenib and propranolol were selected on the basis of previous studies (22, 29).

**Hemodynamic studies.** Hemodynamic evaluation was performed 4 wk after the CBDL or SHAM operation. Under anesthesia with ketamine (100 mg/kg) and midazolam (5 mg/kg), a PE-50 catheter was introduced into the femoral artery and connected to highly sensitive pressure transducer to measure arterial pressure (MAP, mmHg) and heart rate (HR, beats/min). The portal vein was cannulated through an ileocolic vein with another PE-50 catheter and used to measure portal pressure (PP, mmHg).

A nonconstrictive perivascular transit-time ultrasonic flow probe (Transonic Systems, New York, NY) was placed around the superior mesenteric artery and connected to a flowmeter to measure superior mesenteric artery blood flow (SMABF, ml·min⁻¹·100 g⁻¹). Superior mesenteric artery resistance (SMAR, mmHg·ml⁻¹·min⁻¹·100 g⁻¹) was calculated as (MAP – PP)/SMABF (11). A second ultrasonic flow probe was placed around the portal vein, as close as possible to the liver to avoid portal-collateral blood flow, to measure portal vein blood flow (PVBF, ml·min⁻¹·100 g⁻¹).

**Determination of the extent of portal-systemic collateral vessels.** The extent of portal-systemic collateralization was quantified by injection of 51Cr-labeled microspheres (diameter 15 ± 3 μm; specific activity 52.68 mCi/g) into the spleen. Rats were then euthanized with an overdose of ketamine, and radioactivity in liver and lungs was determined in a γ-scintillation counter. This allows quantification of the degree of portosystemic collateralization on a scale from 0 to 100% by the equation collateralization (%) = (lung radioactivity)/(lung radioactivity + liver radioactivity) × 100 (11).

**Histological analysis.** Tissues (mesentery and liver) were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (11). Computer-based quantitative analysis of angio genesis was performed in mesenteric sections with the assistance of Axiovision software (Zeiss) by scanning the entire tissue section and counting areas occupied by vascular structures. We obtained a value from each tissue section and then we averaged results from individual sections in each group (5–8 animals per group).

For semi-quantitative analysis of hepatic fibrosis, liver sections were stained with 0.1% Sirius red, photographed, and analyzed by using a microscope equipped with a digital camera. Four to six fields from each slide were randomly selected and the red-stained area per total area was measured by using AxioVision software. Values are expressed as the mean of fields taken from four to six animals per group. This method of measuring fibrosis, in a recent human study, has been shown to correlate with Ishak scores and hepatic venous pressure gradient (6).

**Immunohistochemistry.** Formalin-fixed paraffin-embedded liver sections from SHAM and CBDL rats were immunostained to determine liver vascularization. Sections were incubated with anti-vWF antibody or phosphate-buffered saline as a negative control. Bound antibody was visualized with dianinobenzidine, as chromogen, and slides were counterstained with hematoxylin. Then, six to ten fields from each slide were randomly selected, and the number of vWF-positive vessels in each field was quantified by use of Axiovision software. Group values are expressed as the mean of 27 to 38 fields taken from three to four rats per group.

**Statistical analysis.** Statistical analysis was performed with SPSS 16.0 package (SPSS, Chicago, IL) and StatsDirect Statistical Software (StatsDirect, Altrincham, UK). Values are expressed as means ± standard deviation and compared with an ANOVA analysis if the variable had a normal Gaussian distribution or the Kruskal-Wallis test if the variable does not follow a normal distribution. Pairwise comparisons were conducted with the least significant difference test (for normally distributed data) or Conover-Inman test (for nonnormal data) as appropriate. The distribution of variables was examined by Shapiro-Wilks test. Differences were considered statistically significant at P values <0.05.

**RESULTS**

In all animals, the 2-wk course of treatment with propranolol, sorafenib, or sorafenib+propranolol was well tolerated, without any sign of toxicity, adverse effects (reduced body weight gain, diarrhea, or hemorrhage), or drug-induced mortality. Rats receiving sorafenib did not show any significant elevation of aspartate aminotransferase (AST) compared with the respective groups receiving vehicle, neither in CBDL rats (241 ± 137 U/l in the sorafenib group vs. 195 ± 22 U/l in vehicle-treated rats) nor in SHAM rats (sorafenib 106 ± 38 U/l vs. 107 ± 76 U/l in the vehicle group).

**Hemodynamic effects.** The main hemodynamic findings in the different groups are summarized in Table 1 and Fig. 1. The CBDL rats treated with vehicle showed a significant increase in PP (108%; P < 0.001), SMABF (63%; P < 0.001), and PVBV (30%; P < 0.001) (Fig. 1) and a reduction of SMAR (60%; P < 0.001) and MAP (25%; P < 0.001) (Table 1), compared...
with SHAM rats treated with vehicle. Portosystemic shunting was negligible in SHAM rats (less than 1%) but extensive (97%) in CBDL rats receiving vehicle (Fig. 1). These hemodynamic data show that the experimental model exhibited the typical hemodynamic abnormalities characteristic of portal hypertension, with hyperdynamic splanchnic circulations.

In SHAM rats, treatment with propranolol decreased significantly PVBF (P < 0.002) (Fig. 1) and HR (P < 0.001), and sorafenib induced a mild reduction in HR (P = 0.038) (Table 1). The combined treatment with propranolol+sorafenib caused significant reductions in PVBF (35%; P = 0.006) (Fig. 1) and HR (11%; P = 0.017) (Table 1) in SHAM rats compared with SHAM rats receiving vehicle. These results are likely to be due to the β-blocker effect of propranolol; in fact they were similar to those obtained with propranolol alone. No differences in PP and MAP values were observed across the different groups of SHAM rats.

In CBDL rats, both propranolol and sorafenib induced a similar reduction in PP and hyperdynamic splanchnic circulation. Indeed propranolol significantly reduced PP by 19% (P <

**Table 1. Additional hemodynamic data in SHAM and CBDL rats**

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<tr>
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<th>SHAM</th>
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<th>CBDL</th>
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<tr>
<td></td>
<td>Vehicle</td>
<td>Propranolol</td>
<td>Sorafenib</td>
<td>Propranolol + Sorafenib</td>
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<tr>
<td><strong>MAP, mmHg</strong></td>
<td>120 ± 9</td>
<td>113 ± 13</td>
<td>111 ± 5</td>
<td>124 ± 12</td>
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<tr>
<td><strong>HR, beats/min</strong></td>
<td>350 ± 24</td>
<td>314 ± 15*</td>
<td>321 ± 21</td>
<td>313 ± 18*</td>
</tr>
<tr>
<td><strong>SMAR, mmHg·mL⁻¹·min⁻¹·100 g body wt⁻¹</strong></td>
<td>43.3 ± 9.9</td>
<td>54.5 ± 9.5</td>
<td>44.9 ± 12.3</td>
<td>53.1 ± 17.8</td>
</tr>
<tr>
<td><strong>Spleen weight, g</strong></td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
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<tr>
<td><strong>Body weight, g</strong></td>
<td>461 ± 15</td>
<td>404 ± 34</td>
<td>391 ± 37</td>
<td>439 ± 19</td>
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Values are means ± SD. SHAM, sham operated; CBDL, common bile duct ligated; MAP, mean arterial pressure; HR, heart rate; SMAR, superior mesenteric artery resistance; K-W, Kruskal-Wallis. *P < 0.05 vs. SHAM vehicle; **P < 0.05 vs. CBDL vehicle; ***P < 0.05 vs. CBDL sorafenib; ****P < 0.05 vs. CBDL propranolol.

![Fig. 1. Hemodynamic effects of propranolol (PROP), sorafenib (SORA), and propranolol+sorafenib on portal pressure, superior mesenteric artery blood flow, portal vein blood flow, and portal-systemic shunting in sham-operated (SHAM) and common bile duct-ligated (CBDL) rats. VEH, vehicle.](http://ajpgi.physiology.org/ by 10.220.33.6 on October 21, 2017 http://ajpgi.physiology.org/ Downloaded from)
0.001), SMABF by 37% (P < 0.001), and PVBF by 35% (P = 0.003) (Fig. 1) and increased the SMAR by 70% (P = 0.025) (Table 1). Sorafenib caused a significant reduction in PP by 15% (P < 0.001) and SMABF by 18% (P = 0.045) (Fig. 1) and increased SMAR by 41% (P = 0.046) (Table 1), whereas PVBF was unchanged (Fig. 1). It follows that whereas propranolol decreased PP by reducing PVBF (−35%), sorafenib decreased PP without decreasing PVBF, reflecting a reduction in intrahepatic resistance.

After sorafenib + propranolol the PP was reduced by 30% (P < 0.001) relative to vehicle, which was significantly greater than the fall achieved with propranolol (−19%; P < 0.001) or sorafenib (−15%; P < 0.001) (Fig. 1). This result confirms that the combination of propranolol + sorafenib has an additive effect reducing PP. In contrast the reductions in SMABF by 45% (P < 0.001) and in PVBF by 39% (P = 0.001) under combined therapy were not significantly greater than those observed after propranolol alone (−37 and −35%, respectively) (Fig. 1).

Effects on splanchnic vascularization. Computer-based quantitative analysis of vascular density in mesenteric tissue sections showed that in CBDL vehicle-treated rats the vascular area was increased by 100% relative to their SHAM control (6.5 vs. 3.2%; P < 0.001) (Fig. 2). Propranolol administration had no effect on splanchnic vascularization in CBDL rats (Fig. 2). In contrast, the administration of sorafenib alone or in combination with propranolol markedly reduced the mesenteric vascular area compared with vehicle-treated rats (−37 and −41%, respectively; P = 0.017) (Fig. 2). No significant effects on splanchnic vascularization were observed in SHAM rats of any treatment group.

Interestingly, in CBDL rats spleen size was also reduced by sorafenib (−10%; not significant) and sorafenib + propranolol (−22%; P = 0.031) (Table 1) relative to vehicle-treated rats, but not by propranolol alone. This is in keeping with our recent results demonstrating that splenomegaly in portal hypertension is due not only to congestion but also to active angiogenesis and fibrogenesis and that splenomegaly can be reduced by an antiangiogenic treatment (28).

Effects on portosystemic collateral circulation. A very high degree (97%) of portosystemic collateralization developed 4 wk after CBDL operation in animals receiving vehicle. Sorafenib, alone or in combination with propranolol, produced a marked and significant reduction in portosystemic shunting compared with vehicle-treated rats (−25%; P = 0.034, and −33%; P = 0.016, respectively) (Fig. 1). This reduction was
mild and not significant after propranolol alone (−5%; not significant) (Fig. 1). Portosystemic collateralization in SHAM groups was negligible (<1%).

**Effects on intrahepatic fibrosis.** To evaluate the possibility that the different treatments could decrease hepatic fibrosis and thus the mechanical component of intrahepatic resistance, liver fibrosis was evaluated by a semiquantitative method. Sirius red-stained area, corresponding to collagen fibers, was measured in liver sections. CBDL resulted in a distortion of the normal liver architecture, with an extensive deposition of fibrillar collagen in portal tracts and central veins (Fig. 3). Sorafenib, alone or associated with propranolol, decreased liver fibrosis in CBDL rats by 38% relative to vehicle-treated rats (sorafenib vs. vehicle \( P = 0.003 \) and propranolol + sorafenib vs. vehicle \( P = 0.005 \)) (Fig. 3). This result is consistent with our previous study (29), in which sorafenib decreased liver fibrosis through an attenuation of the activation of hepatic stellate cells (29). This result suggests that the fall in PP caused by sorafenib was mainly due to a decrease in liver vascular resistance, since portal blood flow was not modified. Propranolol alone had no effect on liver fibrosis (Fig. 3).

**Effects on intrahepatic neovascularization.** The effects of the different treatments on hepatic neovascularization were examined by the immunohistochemical study of vWF protein expression. Development of cirrhosis in CBDL animals was accompanied by a sixfold increase in liver vascularization (from a mean of 4.7 vessels/field in SHAM rats treated with vehicle to 29.6 vessels/field in CBDL rats receiving vehicle) (Fig. 4). Treatment with sorafenib alone decreased liver neovascularization by 42%, whereas sorafenib plus propranolol reduced it by 51% (\( P = 0.04 \) and \( P = 0.02 \) vs. vehicle, respectively) (Fig. 4). Propranolol alone did not significantly decrease liver vascularization (Fig. 4).

**DISCUSSION**

Several studies have shown an improvement of portal hypertension syndrome with different antiangiogenic treatments in animal models (11, 13, 29, 35). Indeed, blockade of the VEGF and PDGF signaling cascade has been shown to translate into a reduction in portal pressure, superior mesenteric artery blood flow, and portosystemic shunting.

A recent study from our laboratory showed that sorafenib, a multikinase inhibitor, decreases PP, improves hyperdynamic syndrome, and decreases portosystemic collateralization and splanchnic inflow in two different animal models of portal hypertension (29). Moreover, treatment with sorafenib was associated with a reduction of inflammation, hepatic stellate...
cell activation, and intrahepatic fibrosis, leading to a decrease in the intrahepatic vascular resistance. These effects of sorafenib on the portal hypertension syndrome have been confirmed by independent studies (20, 31). One of these studies also suggested an effect of sorafenib on the dynamic component of increased intrahepatic vascular resistance mediated by RhoA/Rho kinase (20).

The dose of sorafenib used in the present study (1 mg·kg\(^{-1}\)·day\(^{-1}\)) was chosen according to previous studies performed in our laboratory and proved to decrease angiogenesis and portal pressure in cirrhotic rats (29). At the dose used in this study, sorafenib did not cause toxic effects on the liver, as indicated by the nonexistence of noticeable adverse effects (reduction of body weight gain, diarrhea, hemorrhage), lack of mortality during treatment with sorafenib, and the absence of a significant elevation of AST. This is at variance of what reported by Hennenberg et al. (20), which is likely due to the fact that these authors used a 60-fold-greater dose of sorafenib.

The present study evaluated the hypothesis that blocking angiogenesis by sorafenib may enhance the widely studied beneficial effect of NSBB, which at present are the only drugs recommended for portal hypertension. We hypothesized that by combining propranolol, an agent that reduces portal blood inflow, with sorafenib, which decreases neovascularization, liver fibrosis, and the formation of portosystemic collateral vessels, we would obtain an additive effect on portal pressure and hence a further beneficial effect. The hypothesis was tested by assessing the effect of the association of propranolol and sorafenib in an experimental model of cirrhosis produced by CBDL in rats. This is a fast and very reproducible model of secondary biliary cirrhosis, which proved to be a useful tool for studying portal hypertension (19) that has been widely used in the assessment of new drugs for portal hypertension and liver fibrosis (20, 29, 32). Moreover, studies on the reversion of fibrosis and of angiogenesis using the CBDL model are highly reliable because, in contrast to toxic models of cirrhosis, the noxious stimuli are still present in the animals during the treatment stage, as it happens in many clinical situations.

As previously described in under METHODS, treatments began 2 wk after CBDL or sham operation and were continued for 2 wk. Severe cirrhosis in these rats is achieved 4 wk after intervention. However, by the time that treatment began in the present study, 2 wk after CBDL, rats already show signs of fibrosis and portal hypertension. Keeping in mind the limitations of animal models, this schedule was chosen to match the conditions of therapeutic treatments in patients with cirrhosis, which are usually started when portal hypertension is already
The results of our study clearly demonstrate that both sorafenib and propranolol, given alone for 2 wk to rats with portal hypertension induced by CBDL, cause a significant reduction in portal pressure (−19 and −15%, respectively) compared with vehicle-treated controls. As hypothesized, this reduction in portal pressure was achieved through different mechanisms. Indeed, in CBDL rats propranolol decreased portal pressure by reducing both SMABF (−37%) and PVBF (−35%) with respect to vehicle-treated rats. On the other hand, sorafenib reduced portal pressure mainly by decreasing the intrahepatic vascular resistance, as suggested by the absence of changes in portal blood flow and by the observed reduction in liver fibrosis (−38%). In agreement with our previously reported findings, sorafenib also reduced the formation of portal-systemic collaterals, which explains why the portal blood flow did not decrease despite the slight fall in SMABF and thereby in portal inflow, as the portal blood flow that escaped through the portal-systemic collaterals was markedly decreased after sorafenib.

These findings are not unexpected, since the effectiveness of each drug in decreasing portal hypertension has been well characterized previously (17, 18, 29, 31). However, the most important finding of the present study is that the combined administration of propranolol and sorafenib caused an additive effect decreasing portal pressure (−30%), which matches almost arithmetically the sum of that of either drug given alone (−19% propranolol, −15% sorafenib). It is worth noting that the magnitude of the PP reduction achieved by combination therapy was very pronounced, well above the 20% cutoff decrease that is associated with protection from portal hypertension-related complications in patients with cirrhosis (3, 9, 14). In contrast, the reduction in SMABF and PVBF after combination therapy was not significantly greater than that obtained with propranolol alone. Probably the effect of propranolol on splanchnic blood flow is so marked that it could mask a mild additive effect of sorafenib decreasing SMABF when both drugs were given together.

According with our previous study (29) we confirmed an important inhibitory effect of sorafenib on splanchnic neovascularization when administered alone or in combination with propranolol. On the other hand, treatment with propranolol alone did not show any significant effect on mesenteric vascular density. The combined treatment (sorafenib plus propranolol) also caused a reduction in portal-systemic shunting, slightly greater (−33%) than that observed with sorafenib alone (−25%), whereas propranolol caused only a mild (−5%), nonsignificant decrease in portal-systemic shunting. In addition, sorafenib caused an attenuation of splenomegaly, as shown by a significant decrease in spleen weight (−20%). This change is not observed after treatment with propranolol alone and further reflects the antiangiogenic properties of sorafenib. Although the clinical benefit associated with the reduction in splenomegaly is conjectural, the observed effect on portal-systemic shunting is very significant, since it suggests that the treatment of portal hypertension combining NSBB and low doses of sorafenib may achieve a marked reduction in the formation and extent of esophageal varices in cirrhosis, something that has not been consistently achieved previously by means of pharmacological therapy (17, 18), probably reflecting the fact that it requires a more pronounced decrease in portal pressure, such as that caused by transjugular intrahepatic portosystemic shunt (8, 16).

Vascular structural changes in the cirrhotic liver include vascular remodeling and angiogenesis. The former translates into the capillarization of the hepatic sinusoids, whereas the latter leads to formation of new vessels (25, 34). Most of these newly formed vessels in the cirrhotic liver act as intrahepatic shunts, which probably develop in an attempt to decrease portal pressure (12, 34). On the other hand, these vascular abnormalities may impair the availability of oxygen and nutrients in the liver parenchyma. Consistent with the data obtained in our previous study (29), we currently confirm the important inhibitory effect of sorafenib treatment on liver neovascularization, which was observed both when sorafenib was administered alone or in combination with propranolol. Importantly, this effect goes in parallel with the reduction in liver fibrosis (29, 35). Moreover, although propranolol has shown antiangiogenic effects in several in vivo and in vitro investigations (23, 24), in the present study we have not observed any effect of propranolol on liver or splanchnic neovascularization in CBDL rats.

In conclusion, combined treatment with propranolol and low doses of sorafenib causes a greater reduction in portal pressure than that observed with any of these drugs used alone, which is achieved by a combined effect on splanchnic blood flow and liver resistance. This decrease in portal pressure is accompanied by other beneficial effects, such as a reduction on both liver fibrosis and formation of portosystemic collaterals. Keeping in mind the limitations of translating the results obtained in animal models to clinical practice and the fact that the effectiveness of the combined therapy with propranolol plus sorafenib has only been shown in one model of cirrhosis in the present study (CBDL rats), further studies preferably in other models of cirrhosis should be conducted. Nevertheless, we believe the present findings are clearly encouraging and provide the rationale basis for testing this therapeutic approach in patients with cirrhosis.

Finally, it should be emphasized that the doses of sorafenib used in this study were much lower (on the order of 1/60) than those used in other rat studies (20). It is of interest that the dose in patients with cancer was defined according with maximal tolerability, starting by 400 mg twice per day, whereas probably the dose effective on portal hypertension and liver fibrosis may be much less as suggested by the present findings. This is of relevance since sorafenib at antitumoral doses is associated with a high rate of adverse effects (26). Although no toxic effects were observed in this study, clinical studies to verify this approach in the treatment of portal hypertension, using sorafenib or other antiangiogenic agents, should be extremely cautious and use the minimal effective dose of these compounds.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).
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