Effects of Ritanserin, a Selective and Specific S2-serotonergic Antagonist, on Portal Pressure and Splanchnic Hemodynamics in Rats with Long-term Bile Duct Ligation

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This study investigated the short-term effects of ritanserin, a selective and specific S2-serotonergic antagonist, in an experimental model of cirrhosis and intrahepatic portal hypertension caused by long-term bile duct ligation and division and in normal control rats. The rats subjected to bile duct ligation were randomized under blind conditions into two groups to receive ritanserin (0.7 mg/kg body wt., intravenously; n = 10) or the same volume of placebo (isotonic saline solution; n = 10). We performed hemodynamic studies with radiolabeled microspheres 60 min after drug administration. Two groups of normal rats (n = 6) were studied after they received ritanserin or placebo. Ritanserin administration to rats subjected to bile duct ligation significantly reduced portal pressure (from 16.2 ± 1.3 mm Hg to 12.3 ± 0.7 mm Hg; mean decrease, 22% ± 5%; p < 0.05). This reduction was associated with lower portal venous resistance (4.3 ± 0.5 mm Hg·min·100 gm/ml in the placebo group vs. 3.1 ± 0.3 mm Hg·min·100 gm/ml in rats given ritanserin; mean decrease, 28%; p = 0.069), but we saw no changes in portal vein inflow (3.9 ± 0.5 ml/min·100 gm vs. 4.4 ± 0.4 ml/min·100 gm), mean arterial pressure (110 ± 9 mm Hg vs. 102 ± 5 mm Hg) and cardiac index (32.9 ± 2.5 ml/min·100 gm vs. 40.5 ± 6.7 ml/min·100 gm). Hepatic arterial and kidney blood flows were not modified by ritanserin. Ritanserin had no systemic or splanchnic effects in normal rats. Our results demonstrate that ritanserin infusion decreases portal pressure without any systemic hemodynamic change in rats with secondary biliary cirrhosis and portal hypertension. These findings provide further support for a role of serotonin in the pathogenesis of portal hypertension and suggest a potential use of ritanserin (alone or associated with other agents) in the pharmacological treatment of portal hypertension. (HEPATOLOGY 1993;18:389-393.)

Serotonergic mechanisms were recently suggested to contribute to the pathophysiology of portal hypertension. This suggestion was based on the observation that mesenteric veins from portal-hypertensive rats are hypersensitive to the venoconstrictive effects of serotonin (1) and on the results of studies showing that administration of a variety of serotonin S2-receptor blockers decreases portal pressure in portal hypertension models but not in normal animals (2-5). Ritanserin is a specific and selective S2-receptor antagonist that, unlike other S2-receptor antagonists, is devoid of systemic effects. Ritanserin has been shown to reduce portal pressure in several portal hypertension models (6-8), including CCl4-induced cirrhosis (a rat model of intrahepatic portal hypertension usually associated with minimal extrahepatic portosystemic collaterals) and partial portal vein ligation (PVL) (a prehepatic portal-hypertension model characterized by extensive portosystemic collateral vein formation). In neither model was the fall in portal pressure associated with changes in portal venous inflow, suggesting reduced resistance to portal blood flow. This reduction would occur mainly in the intrahepatic circulation in CCl4-cirrhotic rats and in the collateral veins of rats subjected to PVL. Neither of these models, however, can be extrapolated to the clinical situation because patients with cirrhosis and portal hypertension have prehepatic collateral veins and disturbed intrahepatic circulation.

This study was aimed at characterizing the hemodynamic effects of S-2 antagonism with ritanserin in rats with portal hypertension due to long-term common bile duct ligation and division (CBDL). This is a model of intrahepatic, sinusoidal portal hypertension that, like human cirrhosis, is associated with considerable but quite variable portosystemic collateral vein formation (9-16).

MATERIALS AND METHODS

We studied adult male Sprague-Dawley rats. The animals were housed in individual cages and allowed free access to rat

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chow and water until the time of the study. Secondary biliary cirrhosis with intrahepatic portal hypertension was induced with CBDL in accordance with a previously reported method (10-13). While rats were under ketamine anesthesia (100 mg/kg, intramuscularly), we exposed the common bile duct by median laparotomy and occluded it by double ligature with 5-0 silk thread. The first ligature was made below the junction of the hepatic ducts; the second was made above the entrance of the pancreatic ducts. The common bile duct was then resected between the two ligatures, the abdominal incision was closed and the animals were allowed to recover. Two days after surgery we observed dark-brown urine, indicating successful ligation. Vitamin K (8 mg/kg) was given by intramuscular injection 1 wk before the hemodynamic study. Hemodynamic studies were performed 30 days after surgery. We noted 35% mortality in the 30 days after CBDL; half of the deaths occurred in the first 2 wk after surgery.

Secondary biliary cirrhosis due to long-term CBDL ligation was histologically confirmed in these rats.

Normal control animals were studied after being maintained under the same housing conditions for 30 days.

**Hemodynamic Studies.** The techniques used for hemodynamic measurements have been described (17-20). While rats were under ketamine hydrochloride anesthesia (100 mg/kg body wt, intramuscularly), we cannulated the femoral artery and internal jugular vein with a PE-50 catheter for arterial and right atrial pressure measurements, blood sampling and drug infusion. A midline abdominal incision (2 cm) was made, and the portal vein was cannulated through an ileocolic vein with a PE-50 catheter. After verifying that free reflux of blood had been achieved, we fixed the catheter to the mesentery with cyanoacrylate glue and closed the abdomen with silk sutures. This catheter was used for portal pressure measurements. The left ventricle was catheterized, under pressure monitoring, through the right carotid artery with PE-50 tubing. This catheter was used to monitor heart rate and to inject $^{141}$Ce-labeled microspheres into the left ventricle. All catheters were connected to highly sensitive pressure transducers that were calibrated before each study; blood pressures were registered on a multichannel recorder (model MT6-PX, Lec-tromed, St. Peters, Jersey Channel Islands, UK). The zero point was determined to be 1 cm above the operating table. Rectal temperature was maintained at $37^\circ\pm 0.5^\circ$ C throughout the study.

We measured cardiac output (CO) and regional blood flows with a radioactive microsphere technique described previously (17-22). A reference blood sample was taken from the femoral artery catheter into a preweighed syringe for 75 sec at a rate of 1 ml/min with a continuous-withdrawal pump. Approximately 50,000 microspheres labeled with $^{141}$Ce (15.5 ± 0.1 $\mu$m diameter, specific activity = 10 mCi/gm; Du Pont–New England Nuclear, Boston, MA) were injected into the left ventricle 15 sec after the start of blood withdrawal.

At the end of the experiments, the animals were killed with bolus injections of saturated KCl. The abdominal organs and the kidneys, lungs and testes were dissected, blotted, weighed, cut into small pieces and placed in counting tubes. The radioactivity (counts per minute) of each organ was determined in a gamma-scintillation counter (Packard 800c; Packard Instrument Co., Downers Grove, IL). The interference of chromium radioactivity (energy window = 240 to 400 keV) into the cerium channel (energy window = 100 to 165 keV) was corrected with chromium and cerium standards.

Cardiac output ($CO$) was calculated as

$$CO = \frac{\text{Injected radioactivity (cpm) \times \text{Reference sample blood flow (ml/min)}}}{\text{Reference sample blood radioactivity (cpm)}}$$

Cardiac index ($CI$) was calculated as

$$CI = \frac{\text{CO (ml/min \times 100 gm)}}{\text{CO/body wt (gm) \times 100}}$$

Regional blood flows were calculated as

$$\text{Organ blood flow (ml/min) = \frac{\text{Organ radioactivity (cpm) \times \text{Reference sample blood flow (ml/min)}}}{\text{Reference sample blood radioactivity (cpm)}}$$

Portal vein inflow (PVI), which represents the total blood flow entering the portal venous system, was calculated as the sum of the blood flow to stomach, spleen, small and large intestines, pancreas and mesentry.

Portosystemic shunting (PSS) was estimated as previously described (17, 18, 23) with $^{51}$Cr-labeled microspheres (15.5 ± 0.1 $\mu$m diameter; specific activity = 30.95 mCi/gm) infused into the portal vein through the ileocolic vein catheter as

$$\text{PSS} = \frac{\text{Liver radioactivity} - \text{Lung radioactivity}}{100 \times \text{Lung radioactivity}}$$

Collateral blood flow (CBF) was estimated as

$$\text{CBF (ml/min \times 100 gm)} = \text{PVI \times PSS/100}$$

Portal blood flow (PBF) was calculated as

$$\text{PBF (ml/min \times 100 gm)} = \text{PVI} - \text{CBF}$$

Resistances in the vascular systems were calculated from the ratio between perfusion pressure ($P$) and blood flow ($Q$) of each vascular territory.

In the calculation of the systemic vascular resistance, $P$ was mean arterial pressure minus right atrial pressure and $Q$ was the cardiac output; in the calculation of the splanchic arteriolar resistance, $P$ was the mean arterial pressure minus the portal pressure and $Q$ was the PVI; and in the calculation of the portocollateral and portohepatic resistances, $P$ was the portal pressure minus the right atrial pressure and $Q$ was the CBF or PBF. The vascular resistance of the portal venous system was calculated as portal pressure minus right atrial pressure divided by PVI. This represents the sum of the serial resistances of the portal vein and the hepatic vascular bed and of the parallel resistance of the collateral veins.

The portohepatic gradient was the difference between the portal pressure and the right atrial pressure.

**Experimental Design.** Cirrhotic rats were randomly assigned to one of two groups: Group 1 comprised 10 rats given, through the jugular vein catheter, a bolus of ritanserin (0.7 ml/kg body wt), kindly supplied by Janssen Pharmaceutica (Beerse, Belgium). This dose is known to inhibit completely S2-serotonergic receptors (24). Group 2 was composed of 10 animals given boluses of placebo (an identical amount of isotonic saline solution). The schedule of drug administration was blind. The randomization code was generated by a computer. The code was not revealed until all studies were
Table 1. Comparison of the effects of ritanserin and placebo on splanchnic and systemic hemodynamics in rats subjected to CBDL

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Placebo (n = 10)</th>
<th>Ritanserin (n = 10)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>110 ± 9*</td>
<td>102 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>CI (ml/min · 100 gm body wt)</td>
<td>32.9 ± 2.7</td>
<td>40.5 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic vascular resistance</td>
<td>3.3 ± 0.4</td>
<td>3.1 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>(mm Hg · min · 100 gm body wt/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal pressure (mm Hg)</td>
<td>15.0 ± 0.7</td>
<td>12.3 ± 0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>1.4 ± 0.5</td>
<td>1.8 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>PVI (ml/min · 100 gm body wt)</td>
<td>3.9 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Portal venous system resistance</td>
<td>4.3 ± 0.5</td>
<td>3.1 ± 0.3</td>
<td>0.069</td>
</tr>
<tr>
<td>(mm Hg · min · 100 gm body wt/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal pressure (mm Hg)</td>
<td>6.6 ± 1.0</td>
<td>5.1 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Portalocollateral resistance</td>
<td>18.3 ± 5.2</td>
<td>11.9 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>(mm Hg · min · 100 gm body wt/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splanchnic arteriolar resistance</td>
<td>27.7 ± 4.3</td>
<td>23.3 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>(mm Hg · min · 100 gm body wt/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic arterial blood flow</td>
<td>1.8 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>(ml/min · 100 gm body wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal blood flow (ml/min · 100 gm body wt)</td>
<td>3.2 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>PSS (%)</td>
<td>33.8 ± 7.4</td>
<td>35.0 ± 11.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M.

RESULTS

CBDL. Body weights were similar in the two groups of rats subjected to CBDL: 356 ± 16 gm in the placebo group and 382 ± 15 gm in rats given ritanserin. Similarly, we saw no differences between the two groups in baseline values of portal pressure (16.1 ± 1.1 mm Hg vs. 16.2 ± 1.3 mm Hg), mean arterial pressure (97 ± 7 mm Hg vs. 95 ± 5 mm Hg) and heart rate (306 ± 13 beats/min vs. 318 ± 14 beats/min).

Systemic hemodynamics were not significantly modified by ritanserin (Table 1).

Ritanserin caused a significant reduction in portal pressure, which fell from 16.2 ± 1.3 mm Hg under baseline conditions to 12.3 ± 0.7 mm Hg (−22% ± 5%; p < 0.05). This was not observed in the control (placebo-treated) group. The final portal pressure was significantly lower in the CBDL rats receiving ritanserin than in those receiving placebo (Table 1). The reduction in portal pressure was not associated with changes in PVI.

Portal venous resistance was 28% lower in rats receiving ritanserin than in the placebo group, but this difference was not statistically significant (3.1 ± 0.3 vs. 4.3 ± 0.5 mm Hg · min · 100 gm/ml; p = 0.069). When the two components of the vascular resistance of the portal system—portocollateral resistance and portalocollateral resistance—were considered separately, both were reduced (by 35% and 23%, respectively), although neither change was statistically significant (Table 1).

PSS was similar in CBDL rats receiving ritanserin and placebo (Table 1). Blood flows in each splanchnic organ are shown in Figure 1. Ritanserin did not modify blood flow to the stomach, small intestine, colon, spleen, pancreas or mesentery. Similarly, hepatic arterial blood flow and renal blood flow were not significantly different between CBDL rats receiving placebo or ritanserin (Table 1).

Normal rats. The normal rats receiving placebo (Table 2) had negligible PSS and lower portal pressure, PVI and CI than did control CBDL rats, whereas mean arterial pressure and systemic vascular resistance were higher, thus confirming that the systemic and splanchnic circulations were hyperkinetic in portal-hypertensive CBDL rats.

Neither the systemic nor the splanchnic circulation was modified by ritanserin administration in normal rats (Table 2).

DISCUSSION

This study was aimed at investigating the effects of ritanserin in an experimental model of intrahepatic portal hypertension due to secondary biliary cirrhosis.
TABLE 2. Comparison of effects of ritanserin and placebo on splanchnic and systemic hemodynamics in normal rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Placebo (n = 6)</th>
<th>Ritanserin (n = 6)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>122 ± 10*</td>
<td>120 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>CI (ml/min · 100 gm body wt)</td>
<td>24.0 ± 1.3</td>
<td>28.1 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic vascular resistance (mm Hg · min · 100 gm body wt/ml)</td>
<td>5.2 ± 0.6</td>
<td>4.5 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Portal pressure (mm Hg)</td>
<td>8.7 ± 0.8</td>
<td>8.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>PVI (ml/min · 100 gm body wt)</td>
<td>2.4 ± 0.5</td>
<td>3.1 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Portal venous system resistance (mm Hg · min · 100 gm body wt/ml)</td>
<td>3.9 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>PSS (%)</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data expressed as mean ± S.E.M.

FIG. 1. Blood flows of splanchnic organs in portal-hypertensive rats receiving ritanserin or placebo.

caused by CBDL. Unlike other experimental models, the extent of spontaneous PSS in CBDL rats, although variable, is usually moderate (9, 12) (mean value = 35%), as seen in patients with alcoholic cirrhosis (15, 16). In this model, changes in vascular resistance to portal blood flow reflect changes in collateral and intrahepatic vascular resistance. Because the effects of antiserotoninergic drugs are thought to be mediated by reduction of vascular resistance to portal blood flow (5-8), studies in this model can be useful in assessing the potential beneficial effects of ritanserin in portal hypertension.

The results of this study reveal that ritanserin induced a significant reduction in portal pressure in rats with CBDL. This finding was observed both on evaluation of changes in portal pressure 60 min after injection (-22% ± 5%) and also when the group subjected to CBDL and treated with ritanserin was compared with a group of control rats subjected to CBDL and treated with the same volume of saline solution under blind conditions.

The mechanism by which S2-receptor blockade with ritanserin decreased portal pressure is not entirely defined, although the fact that no reduction in portal blood inflow occurred strongly suggests that ritanserin lowers portal pressure by decreasing resistance to PBF. Actually, the change in overall portal venous resistance (average = 28%) was close to statistical significance (p = 0.069). Furthermore, our findings suggest that resistance to PBF occurred in the portocollateral circulation (which decreased by 35%) and in the portohepatic circulation (which decreased by a mean of 23%). These observations are in accordance with the results of previous studies showing that ritanserin lowers portal pressure without modifying portal inflow in CCl4-cirrhotic rats (6) (in which increased resistance to PBF occurs in the hepatic circulation, whereas formation of portosystemic collateral veins is usually mild) and in rats subjected to partial PVL (8), in which increased resistance occurs at the ligature of the portal vein and along the extensive portal systemic collateral veins that characterize this model.

The reduction by ritanserin of portal pressure and portocollateral resistance may be of therapeutic potential in patients with portal hypertension. It is interesting to note that, in patients with cirrhosis, the greatest portal pressure reductions are observed on combination of the administration of a vasoconstrictive drug reducing portal inflow (such as vasopressin or propranolol) with a venous dilator (such as nitroglycerin or isosorbide-5-mononitrate) (25-27). Our finding that ritanserin decreases portal resistance without causing systemic hypotension (6-8) suggests that it would be a useful adjunct in the pharmacological treatment of portal hypertension.

On the other hand, the fact that ritanserin had no effect on arterial pressure, CI or peripheral resistance provides indirect evidence that ritanserin, unlike ketanserin (2-4, 28, 29), is highly selective for S2 receptors. This is in keeping with results of in vitro studies showing that the affinity of ritanserin for S2 receptors is 200 times higher than for α1-adrenergic receptors (30).

Previous studies (6) have shown that ritanserin, a selective and specific blocker of serotonin-S2 receptors (which are present in the portal vein of the rat) (31, 32), has no effect on portal pressure in normal rats. This has been confirmed in this study; we found no effect of ritanserin in the splanchnic and systemic circulations of
normal control rats. Therefore our findings in rats subjected to CBDL further support the suggestion that serotonergic mechanisms are at work in the pathogenesis of portal hypertension. Blockade of S2 receptors with ritanserin, alone or in combination with other drugs, may be of value in the treatment of portal hypertension.

REFERENCES


