Lanreotide on Collateral Response to Endothelin-1 and Vasopressin in Cirrhotic Rats

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Introduction

Gastroesophageal variceal bleeding is a severe complication in cirrhotic patients. The vascular hyporesponsiveness to vasoconstrictive agents during hemorrhage also compromises hemostasis.1,2 Somatostatin and its long-acting analog octreotide have been advocated to control variceal hemorrhage with mechanisms not fully elucidated.3,4 They seem to reduce splanchnic blood flow mainly through the diminution of vasoactive hormone release.5 Long-term octreotide treatment has been found to ameliorate hyperdynamic circulation and prevent in vitro mesenteric vascular hyporeactivity in partially portal-vein-ligated (PVL) portal hypertensive rats.6 In addition, it decreased portal pressure and enhanced the constrictive response to vasopressin in mesenteric arteries of PVL rats.7 Long-term octreotide application also reduced portal pressure in cirrhotic rats.8 However, the requirement of repeated injection and subsequent desensitization9,10 hamper its clinical utilization and therapeutic efficacy.

Endothelin-1 (ET-1), an endothelium-derived peptide with protein kinase C-related vasoconstrictive properties,11 has been found to be increased in the liver of cirrhotic patients, and its level is proportional to the severity of liver disease.12 The decreased metabolism and enhanced synthesis of ET-1 in hepatocytes account for its elevated level in cirrhosis.13

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ET-1 induces intrahepatic and portosystemic collateral vasoconstriction in cirrhotic rats and PVL rats. In addition, somatostatin and octreotide enhance ET-1-related portosystemic collateral vasoconstriction if administered in the perfusate before ET-1 incubation. However, the effect of chronic somatostatin analog administration on the collateral vascular ET-1 responsiveness in cirrhotic status is unknown.

Vasopressin controls gastroesophageal varical hemorrhage mainly through splanchnic vasoconstriction with decreased portal venous inflow and portal pressure. Previous study indicated that vasopressin decreased blood flow velocities of superior mesenteric artery, portal vein, and esophageal varices. Furthermore, vasopressin exerts direct vasoconstrictive effect on portosystemic collaterals of PVL rats. It has been indicated that long-term octreotide administration did not enhance the collateral vasoconstrictive effect to vasopressin in PVL rats. However, whether or not somatostatin analogs augment collateral vascular responsiveness to vasopressin in cirrhosis deserves further evaluation.

Considering the inconvenience of octreotide in chronic treatment and the scarcity of information regarding the actions of its related compounds on collateral vascular responsiveness to vasoconstrictors, this study used the in situ perfusion model to assess the effect of lanreotide, a longer-acting somatostatin analog when compared with octreotide, on the collateral vascular reaction to ET-1 and arginine vasopressin (AVP) in bile duct-ligated cirrhotic rats.

The incidence was then closed and the animal was allowed to recover. According to the literature, a high yield of secondary biliary cirrhosis was noted 5 weeks after BDL. To avoid coagulation defects, BDL rats received vitamin K injection weekly (50 µg/kg, intramuscularly). Perfusion studies were performed 6 weeks after BDL under ketamine anesthesia. Body weights were measured on the day of perfusion studies. The experiments adhered to the American Physiological Society’s guiding principles for the care and use of laboratory animals.

In situ perfusion preparation

The system was operated as previously described. Both jugular veins were cannulated with 16-gauge Teflon cannulas to ensure an adequate outflow without any resistance even at the highest flow rates. Heparin (200 U/100 g body weight) was injected through one of the cannulas. The abdomen was then opened and an 18-gauge Teflon cannula was inserted in the distal superior mesenteric vein and fixed with cyanoacrylate glue. To exclude the liver from perfusion, a ligature was tied around the portal vein. The animal was transferred into the upper compartment of a warm chamber (37±0.5°C). The temperature around the perfusion area was continuously monitored with a thermometer inside the mesentry and maintained at 37±0.5°C with a thermostatic pad and temperature-controlled infrared lamp. An open circuit perfusion was then started with Krebs solution (composition in mmol/L: NaCl, 118; KCl, 4.7; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 2.5; NaHCO3, 25; dextrose, 11.0; pH, 7.4; 37±0.5°C) via the mesenteric cannula by a roller pump (model 505S; Watson-Marlow Limited, Falmouth, Cornwall, UK). The perfusate was equilibrated with carbogen gas (95% O2–5% CO2) by a silastic membrane lung. Bilateral jugular vein cannulas were opened to allow a complete washout of blood. Pneumothorax was created by opening slits through the diaphragm to increase pulmonary artery resistance and prevent the perfusate from entering the left chambers of the heart. The collaterals were then perfused with oxygenated (95% O2–5% CO2) Krebs solution containing 3% wt/vol albumin (factor V bovine serum albumin; Sigma Chemical Co, St. Louis, MO, USA). The effluent of the perfused tissue was collected in a reservoir in the lower compartment of the chamber and not recirculated. To continuously monitor and record the collateral pressure, a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA) attached to a Gould model RS 3400 recorder (Gould Inc., Cupertino, CA, USA) was connected to a side arm placed just proximal to the perfusion cannula, with the zero placed at the level of the

Methods

Animal model

Male Sprague–Dawley rats, weighing 240–270 g at the time of surgery, were used. They were housed in plastic cages and allowed free access to food and water, and then fasted for 12 hours before operation. Secondary biliary cirrhosis was induced by common bile duct ligation (BDL) with formalin infusion. Under ketamine anesthesia (100 mg/kg, intramuscularly), the common bile duct was exposed through a midline abdominal incision. It was catheterized by a PE-10 catheter and doubly ligated with 3-0 silk. The first ligature was below the entrance of the pancreatic duct. Ten percent formalin (~100 µL/100 g) was slowly injected into the biliary tree to prevent the subsequent dilatation of the ligated residual bile duct. The PE-10 catheter was then removed and the ligatures tightened, followed by section of the common bile duct between the ligatures.
right atrium. Because the temperature and pressure of the system were stabilized within 20 minutes, all the experiments were performed 25 minutes after starting perfusion at a constant rate of $12 \text{ mL/minute}$.16 Moreover, the perfusion flow rate was kept constant throughout the whole experiment, so the perfusion pressure changes reflected the collateral vascular resistance changes. Only 1 concentration–response curve was performed in each preparation. In each preparation, after testing experimental agents, the contracting capability was challenged with a $125 \text{ mmol/L}$ potassium chloride solution at the end of the experiments.

**Measurement of systemic and portal hemodynamics**

The right femoral artery and mesenteric vein were cannulated with PE-50 catheters connected to a Spectramed DTX transducer (Spectramed Inc.). Continuous recordings of mean arterial pressure, heart rate, and portal pressure were performed on a multichannel recorder (model RS 3400, Gould Inc.). The external zero reference was placed at the level of the midportion of the rat.28,29

**Experimental design**

BDL rats randomly received lanreotide (10 mg/kg, intramuscularly; Sandostatin® LAR®, Novartis, Basel, Switzerland) or distilled water (control) once on the 33rd day after ligation. On the 43rd day after BDL, rats underwent ketamine anesthesia, and then the body weight, mean arterial pressure, portal pressure, and heart rate were measured. Two series of experiments with the in situ collateral perfusion model were performed. In the first series (lanreotide, $n=6$; control, $n=7$), cumulative concentration–response curves were determined by graded final concentrations of ET-1 ($10^{-10}$, $10^{-9}$, $3 \times 10^{-9}$, $10^{-8}$, $3 \times 10^{-8}$, $10^{-7}$ mol/L, respectively) with a constant flow rate ($12 \text{ mL/minute}$).16 In the second series, the same procedure was performed for another 2 groups of BDL rats (lanreotide, $n=6$; control, $n=8$) with $10^{-10}$, $10^{-9}$, $3 \times 10^{-9}$, $10^{-8}$, $3 \times 10^{-8}$, $10^{-7}$, and $3 \times 10^{-7}$ mol/L of AVP, respectively. Each new concentration was allowed to stabilize for 3 minutes before the next higher concentration was added.

**Drugs**

Lanreotide (Sandostatin® LAR®) was purchased from Norvatis Co. (Basel, Switzerland). The reagents for preparing Krebs solution, ET-1, and AVP were purchased from Sigma Chemical Co. All solutions were freshly prepared on the days of the experiments.

**Data analysis**

All results are expressed as mean±SEM. The changes in perfusion pressure (mmHg) over baseline were calculated for each concentration in each preparation. Statistical analyses were performed using a 2-sample $t$ test. Results were considered statistically significant at a 2-tailed $p$ value of less than 0.05.

**Results**

**Hemodynamic effects of lanreotide**

Table 1 shows the body weights and baseline hemodynamic parameters of the different groups. They were similar between the lanreotide and control groups.

**Concentration–response relationships to ET-1**

Figure 1 depicts the concentration–response curves to various concentrations of ET-1 at a constant flow rate. The maximal pressure changes occurred at $10^{-7}$ mol/L. Compared with the control group, the lanreotide group had higher perfusion pressure changes, which reached statistical significance at the ET-1 concentration of $10^{-7}$ mol/L (lanreotide vs. control: perfusion pressure change, $25.2 \pm 1.7 \text{ mmHg}$ vs. $19.6 \pm 1.7 \text{ mmHg}$, $p=0.032$; percentage change: $182.8 \pm 9.5\%$ vs. $132.1 \pm 15.0\%$, $p=0.019$). The maximal pressure change

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**Table 1. Body weight and baseline hemodynamics in the different experimental groups**

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PP (mmHg)</th>
<th>Baseline PP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1 study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lanreotide</td>
<td>6</td>
<td>367.7±7.6</td>
<td>85.0±7.4</td>
<td>257.0±21.1</td>
<td>14.9±1.5</td>
<td>13.8±0.7</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>349.3±9.4</td>
<td>104.2±5.3</td>
<td>278.1±21.6</td>
<td>16.6±1.3</td>
<td>13.6±0.6</td>
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<tr>
<td>AVP study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lanreotide</td>
<td>6</td>
<td>376.3±20.3</td>
<td>92.3±5.7</td>
<td>248.3±13.8</td>
<td>14.7±1.2</td>
<td>16.3±0.8</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>403.0±16.5</td>
<td>94.9±5.8</td>
<td>268.9±19.8</td>
<td>16.6±1.2</td>
<td>14.5±1.6</td>
</tr>
</tbody>
</table>

$p<0.05$ between lanreotide and control groups. BW = body weight; MAP = mean arterial pressure; HR = heart rate; PP = portal pressure; Baseline PP = baseline perfusion pressure; ET-1 = endothelin-1; AVP = arginine vasopressin.
of the portosystemic collateral vessels challenged with the 125 mmol/L potassium chloride solution at the end of experiments was 26.0 ± 3.1 mmHg for the lanreotide group, which was not significantly different from the control group (24.0 ± 1.8 mmHg, \( p = 0.572 \)).

**Discussion**

Lanreotide, a somatostatin analog with a particular ligand affinity for somatostatin receptor subtypes SSTR-2 and -5, has been developed and requires only weekly injections. \(^{30}\) Continuous lanreotide infusion attenuates postprandial splanchnic hyperemia in both normal subjects \(^{31}\) and cirrhotic patients. \(^{32}\) In a previous study, no diminishing effect of lanreotide was found over 8 hours of constant food stimulation, i.e. no downregulation or tachyphylaxis was observed. \(^{33}\) This distinct feature makes long-term lanreotide administration possible without significantly compromised efficacy. Since repetitive and short-term injections hinder the clinical feasibility of somatostatin analogs, we surveyed the effect of lanreotide on collateral vascular response with the

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**Figure 1.** Concentration–response curves to endothelin-1 (ET-1) in portosystemic collateral vascular beds of lanreotide and distilled-water (control)-treated BDL rats, expressed as (A) absolute and (B) percentage increases over baseline value.

**Figure 2.** Concentration–response curves to arginine vasopressin (AVP) in portosystemic collateral vascular beds of lanreotide and distilled-water (control)-treated BDL rats, expressed as (A) absolute and (B) percentage increases over baseline value.
longest applicable duration from drug administration to perfusion studies. According to Kuhn et al., plasma lanreotide concentrations in healthy males remain above 1.5 ng/mL until day 11. Therefore, we decided to inject lanreotide on the 33rd day and performed perfusion study on the 43rd day after BDL.

Lanreotide did not modify mean arterial pressure in this study, consistent with the result of lanreotide injection once per week for 3 weeks in cirrhotic patients. The somatostatin-agonist-dependent desensitization and internalization of the receptor SST2 was ascribed to play a role in the attenuation in depot lanreotide’s hemodynamic efficacy. It has been found that in portal hypertensive rats, a single intramuscular injection of lanreotide 10 mg/kg immediately after PVL did not induce significant changes in cardiac index and systemic vascular resistance on the 8th day after PVL, though significant alterations were noted on the 4th day. In patients with cirrhosis, octreotide 250 μg/12 hr subcutaneously for 5 days exerted no significant changes in systemic hemodynamics. In addition, an immediate but short-acting influence of octreotide on mean arterial pressure of cirrhotic patients was observed, which lasted for only 5 minutes. Therefore, somatostatin analogs do not induce sustained systemic hemodynamic changes, both in portal hypertensive and cirrhotic states.

The influence of somatostatin analogs on the portal system is under debate. It has been demonstrated that acute octreotide injection decreased portal tributary blood flow and portal pressure in portal hypertensive and cirrhotic rats. A similar finding was also found in cirrhotic patients with acute treatment. However, continuous infusion of octreotide in cirrhotic patients failed to show such a beneficial effect. In a study with intravenous bolus of octreotide followed by continuous infusion in cirrhotic patients, portal venous flow decreased compared to baseline at 1 minute but rapidly returned toward baseline, and by the 5th minute, no significant difference was noted. Controversies also exist in chronic treatment. In cirrhotic patients and rats, long-term octreotide administration exerts a portal hypotensive effect, whereas the same finding was not observed in rats with BDL or carbon tetra-chloride-induced cirrhosis.

Portal pressure was not altered by lanreotide in the current study. In cirrhotic patients, lanreotide SR 30 mg once weekly for 3 weeks did not reduce baseline and postprandial hepatic venous pressure gradient or portal blood flow. However, these results are inconsistent with those of a previous study, which indicated that 1 lanreotide injection decreased portal pressure and portal tributary blood flow in PVL rats. The major cause of discrepancy may lie in the different models, lanreotide application schedules and timing of hemodynamic measurements. In the present study, to simulate the clinical situation, lanreotide was administered on the 33rd day after BDL, after the initiation of hepatic injury and cirrhosis. In addition, hemodynamic measurements were performed on the 10th day after lanreotide injection. However, in the previous study, lanreotide was injected immediately after PVL and hemodynamic changes were evaluated on the 4th and 8th days after PVL, respectively. Furthermore, collateral vascular response to ET-1 was enhanced by lanreotide in the current study, which may contribute to a higher portal pressure and offset the potential portal hypotensive effect of lanreotide.

Apart from differences in duration and dosage of octreotide treatment and the time of hemodynamic survey, in cirrhotic rats receiving 1-week octreotide, increased levels of serum 6-keto-PGF1α, nitrite/nitrate, and enhanced aortic endothelial nitric oxide synthase expression without correction of the hemodynamic derangement were observed. Accentuated endothelium-related vasodilatory activity after octreotide treatment may overcome the octreotide-induced hemodynamic effects in cirrhosis. Furthermore, it has been demonstrated that octreotide caused a marked and transient decrease in portal pressure and aygos blood flow and an increase in mean arterial pressure in cirrhotic patients. These effects lasted for only 5 minutes, despite addition of continuous octreotide infusions. Repeated boluses even caused tachyphylaxis. Resistance to octreotide by receptor adaptation during 1-week octreotide administration was also impressed. This rapid desensitization to the effects of octreotide may explain its divergent hemodynamic impacts in cirrhosis.

The effect of somatostatin analogs on variceal pressure is still controversial, including a modest decrease or no modification. It has been noted that octreotide decreased hepatic and aygos blood flows but exerted only minimal effects on portal pressure. In addition, early and chronic administration of octreotide prevented the development of portocollateral blood flow without reducing portal pressure in 2 rat models of cirrhosis. These observations suggest the possibility of an increase in portal collateral resistance. Although somatostatin and octreotide had no direct vasoconstrictive effect on the collateral vessels of PVL rats, in the presence of ET-1, somatostatin and octreotide added in perfusate enhanced the ET-1-induced collateral vasoconstriction. This is compatible with the finding that in the presence of vasoconstrictors involving activation of protein kinase C, such as ET-1,
octreotide exerts a local vasoconstrictive effect on the superior mesenteric artery. Similarly, potassium chloride (KCl)-induced vasoconstriction was not potentiated by octreotide in the mesenteric vascular beds of PVL rats. In the current study, a single injection of lanreotide 10 days before perfusion study exerted improved collateral vascular contraction response to ET-1 at the concentration of $10^{-7}$ mol/L, whereas it did not enhance the contractile response to KCl. In parallel, octreotide administration for 7 days did not change the collateral vascular responsiveness to KCl. Since ET-1 plays a pivotal role in the pathophysiology of portal hypertension, the lack of a portal hypotensive effect of lanreotide may be partially related to the enhanced collateral vascular response to ET-1 by lanreotide. On the other hand, lanreotide did not induce changes of collateral vascular responsiveness to AVP, similar to a previous report on chronic octreotide treatment. This might be attributed to the fact that vasopressin does not act via protein kinase C.

In conclusion, a single injection of lanreotide, a long-acting somatostatin analog, enhances portosystemic collateral vascular responsiveness to ET-1 in BDL cirrhotic rats. However, it does not influence the hyperdynamic circulation or the collateral reactivity to vasopressin.

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