Chronic Indomethacin Treatment Enhances the Portal-systemic Collateral Vascular Response to Vasopressin in Bile-duct Ligated Rats

Hui-Chun Huang1,4, Sun-Sang Wang3,4, Ching-Chih Chang3,4, Fa-Yauh Lee1,2,4*, Full-Young Chang1,4, Han-Chieh Lin1,4, Ming-Chih Hou1,4, Rei-Hwa Lu1, Shou-Dong Lee1,4

Divisions of 1Gastroenterology and 2General Medicine, Department of Medicine, Taipei Veterans General Hospital, 3Taipei Municipal Gan-Dau Hospital, and 4National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.

Background: Liver cirrhosis is often accompanied by portal-systemic collateral formation with hemorrhage and encephalopathy. Prostacyclin participates in hyperdynamic circulation and vascular hyporeactivenss to vasoconstrictors in cirrhosis. It has been shown that arginine vasopressin (AVP) induces direct collateral vasoconstriction in portal hypertensive rats, which is potentiated by indomethacin preincubation. However, the influence of chronic indomethacin administration in cirrhosis remains unexplored.

Methods: This study was performed on male Sprague-Dawley rats with liver cirrhosis induced by common bile duct ligation. They received subcutaneous indomethacin (5 mg/kg/day) or distilled water (control) injection from the 36th to 42nd day after operation. On the 43rd day, systemic and portal hemodynamics were evaluated and the following experiments were performed with an in situ collateral perfusion model: in the first series, concentration-response curves to AVP (10−10−10−7 M) were obtained; in the second series, flow-pressure curves were plotted (Krebs solution, 6–18 mL/min), where the slope represents an index of collateral vascular resistance (the higher the resistance, the smaller the amount of shunting vessels, that is, the lower the degree of shunting).

Results: The mean arterial pressure and portal pressure were similar between indomethacin and control groups (p > 0.05). Indomethacin elevated the collateral perfusion pressure to AVP (3 × 10−9, 10−8 M, p < 0.05) but did not influence the slope of the flow-pressure curve (p > 0.05).

Conclusion: In bile duct-ligated cirrhotic rats, indomethacin improves the portal-systemic collateral vascular responsiveness to AVP without alleviating the severity of shunting. [J Chin Med Assoc 2007;70(12):521–526]

Key Words: indomethacin, liver cirrhosis, portal-systemic collaterals, vasopressin

Introduction

Liver cirrhosis with portal hypertension is one of the most disastrous conditions related to chronic hepatic injury. As portal pressure elevates, portal-systemic collaterals develop gradually to diverse blood flow from the portal system.1 Rupture of the collaterals, especially gastroesophageal varices, leads to high morbidity and mortality rates. To control variceal hemorrhage, vasopressin has been used in past decades by virtue of its splanchnic vasoconstrictive effect with subsequently decreased portal venous inflow and portal pressure.2 In addition, vasopressin exerts a direct vasoconstrictive effect on portal-systemic collaterals.3 However, previous studies in portal hypertensive animals found worse splanchic vascular contractile response to vasoconstrictors than that of normal ones.4,5 Survey on portal hypertensive animals and cirrhotic patients further demonstrated a less prominent portal hypotensive effect of vasopressin during acute hemorrhage as compared with that during

*Correspondence to: Dr Fa-Yauh Lee, Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: fylee@vghtpe.gov.tw • Received: July 20, 2007 • Accepted: October 29, 2007
stable condition,\textsuperscript{6,7} that is, the so-called splanchnic hyposensitivity.

It has been reported that indomethacin attenuates vascular hyposensitiveness to various vasoconstrictors\textsuperscript{4,8} associated with portal hypertension, suggesting the participation of prostanoids in the mechanism. The ability of indomethacin to ameliorate splanchnic hyposensitiveness to terlipressin, a long-acting vasopressin analog, in hemorrhage-transfused cirrhotic rats further supports its role in splanchnic hyposensitivity of cirrhotic rats during acute hemorrhage.\textsuperscript{8} In the collateral vascular bed, the contractile reactivity to vasopressin is also enhanced by indomethacin preincubation in rats with prehepatic portal hypertension and a relatively high degree of portal-systemic shunting.\textsuperscript{3} However, the effects of long-term indomethacin administration in cirrhotic rats with a relatively lower number of collaterals remain to be clarified. Therefore, we conducted this study to survey the influences of chronic indomethacin use on portal-systemic collateral vascular response to vasopressin and the degree of shunting in bile-duct ligated rats.

**Methods**

**Experimental design**

Two groups of rats that underwent bile-duct ligation (BDL) received either indomethacin (5 mg/kg subcutaneous injection, qd) or distilled water (control) from the 36th to the 42nd day after operation. On the 43rd day after BDL, rats were anesthetized with ketamine hydrochloride (100 mg/kg intramuscularly), and the body weight, mean arterial pressure, portal pressure, and heart rate were measured.\textsuperscript{9} Two series of experiments with the in situ collateral vascular perfusion model were performed. In the first series (control, \(n=8\); indomethacin, \(n=8\)), cumulative concentration-response curves of collateral vessels were determined by graded final concentrations of arginine vasopressin (AVP) in escalation with a constant flow rate (12 mL/min). The final concentrations in perfusate were from \(10^{-10}\) to \(10^{-7}\) mol/L of AVP in perfusate. Each new concentration was allowed to stabilize for 3 minutes before the next higher concentration was added. In the second series (control, \(n=7\); indomethacin, \(n=9\)), flow-pressure curves were obtained with Krebs solution to assess the collateral vascular resistance (the slope of the curve represents an index of portal-systemic shunting; that is, the higher the slope [resistance], the lower the relative number of portal-systemic shunting vessels).\textsuperscript{10} The flow rates were 6, 9, 12, 15 and 18 mL/min, respectively.

**Animal model**

Male Sprague-Dawley rats weighing 240–270 g at the time of surgery were used for experiments. The rats were housed in plastic cages and allowed free access to food and water. All rats were fasted for 12 hours before the operation. In all experiments, the authors adhered to the American Physiological Society Guiding Principles for the Care and Use of Laboratory Animals. Rats with secondary biliary cirrhosis were induced with BDL followed by injecting formalin into the biliary tree.\textsuperscript{11} Under ketamine anesthesia (100 mg/kg, intramuscularly), the common bile duct was exposed through a midline abdominal incision, catheterized by a PE-10 catheter, and doubly ligated with 3-0 silk. The first ligature was made below the junction of the hepatic ducts and the second ligature above the entrance of the pancreatic duct. Then, 10% formalin (\(~100\mu L/100\) g body weight) was slowly injected into the biliary tree above the first ligature 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. The rats were allowed to recover. A high yield of secondary biliary cirrhosis were noted 5 weeks after the ligation.\textsuperscript{12,13} To avoid coagulation defects, BDL rats received weekly vitamin K injections (50µg/kg, intramuscularly).\textsuperscript{13}
preparation, the contracting capability was challenged with a 125-mmol/L potassium chloride solution at the end of experiments.

Measurement of systemic and portal hemodynamics
The right femoral artery was cannulated with a PE-50 catheter that was 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 mid-portion of the rat. The abdomen was then opened with a midline incision, and a mesenteric vein was cannulated with a PE-50 catheter connected to a Spectramed DTX transducer. The abdominal cavity was closed and the portal pressure recorded on the Gould model RS 3400 recorder.17

Drugs
Indomethacin, the reagents for preparing Krebs solution, 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 ± standard error of the mean. The changes in perfusion pressure (mmHg) over baseline were calculated for each concentration in each preparation. The concentration of AVP exhibiting 50% of the maximal response (EC50) in each preparation was calculated from sigmoid logistic curves and expressed as negative log molar (–logM). The flow-pressure curves were analyzed by linear regression. Statistical analyses were performed using independent Student’s t test. Results were considered to be statistically significant at a 2-tailed p value of less than 0.05.

Results
Hemodynamic effects of indomethacin
Table 1 shows the body weights and baseline hemodynamic parameters of the indomethacin-treated and control groups in 2 series of studies. The body weights, mean arterial pressure, portal pressure, heart rates and baseline perfusion pressure were similar between the indomethacin and control groups in the first and second series (p > 0.05).

Concentration-response relationships to AVP
Figure 1 depicts the concentration-response curves obtained at a constant perfusion flow rate by the cumulative addition of AVP into the perfusate for the 2 groups. As compared with the control group, the indomethacin-treated group showed significantly higher perfusion pressure to AVP at the concentration of 10^-8 (indomethacin vs. control: 12.8 ± 1.7 vs. 7.5 ± 1.2 mmHg, p = 0.02) and 3 × 10^-9 (7.6 ± 0.9 vs. 4.8 ± 0.8 mmHg, p = 0.027). The maximal perfusion pressure change occurred at the concentration of 3 × 10^-8 M. There was no significant difference in EC50 between the 2 groups (indomethacin vs. control [–logEC50]: 8.73 ± 0.41 vs. 7.95 ± 0.48, p = 0.253). The maximal pressure change of the portal-systemic collateral vessels challenged with the 125-mmol/L potassium chloride solution at the end of experiments was 22.9 ± 2.8 mmHg for the indomethacin-treated group, higher than that of the control group but without statistical significance (19.1 ± 2.8 mmHg, p = 0.347).

Flow-pressure relationships
Figure 2 shows the flow-pressure relationship in the perfused portal-systemic collaterals of indomethacin-treated and control rats. The slope of the indomethacin-treated group was not significantly different from that of the control group (indomethacin vs. control: 0.99 ± 0.07 vs. 0.94 ± 0.04, p = 0.604).

Table 1. Body weights and baseline hemodynamics in different groups*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PorP (mmHg)</th>
<th>Baseline PerP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration-response curve study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INDO</td>
<td>8</td>
<td>382 ± 14</td>
<td>90 ± 6</td>
<td>260 ± 12</td>
<td>15.7 ± 1.0</td>
<td>16.1 ± 0.9</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>346 ± 10</td>
<td>86 ± 4</td>
<td>254 ± 12</td>
<td>14.8 ± 0.4</td>
<td>15.4 ± 1.2</td>
</tr>
<tr>
<td>Flow-pressure curve study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INDO</td>
<td>9</td>
<td>363 ± 18</td>
<td>82.9 ± 3</td>
<td>258 ± 12</td>
<td>12.7 ± 0.6</td>
<td>15.7 ± 0.6</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>366 ± 20</td>
<td>81.6 ± 5</td>
<td>254 ± 12</td>
<td>14.4 ± 0.4</td>
<td>17.3 ± 1.3</td>
</tr>
</tbody>
</table>

*There was no significant difference between the INDO and control groups. BW = body weight; MAP = mean arterial pressure; HR = heart rate; PorP = portal pressure; Baseline PerP = baseline perfusion pressure; INDO = indomethacin.
Indomethacin is a potent inhibitor of the cyclooxygenases in the synthesis of various prostaglandins and has been surveyed in various pathologic conditions. Many investigations find that the hyperdynamic circulation of portal hypertension can be attenuated by indomethacin. However, the systemic and portal hemodynamics did not reach remarkable changes in the present study. A similar finding has been reported with daily injection of indomethacin (4 mg/kg/body weight) for 2 days in rats after partial portal vein ligation. The actual reason for the contradictory results is not clear and might be due to different experimental designs. However, vascular homeostasis maintained by endogenous vasoactive substances might be considered.

For instance, unaltered blood pressure due to reciprocal interrelationship between nitric oxide (NO) and prostaglandins has been observed and indomethacin enhances interleukin-1β-induced nitrite release.

In a previous study, indomethacin incubation significantly potentiated the response of portal-systemic collaterals to AVP in portal hypertensive rats. The enhanced response to AVP by indomethacin suggested the role of prostaglandin in the regulation of the portal-systemic collateral circulation. We also found in the current study that long-term use of indomethacin improved the collateral vascular responsiveness to AVP in BDL cirrhotic rats. Gastroesophageal varices are the most prominent portal-systemic collaterals in cirrhotic patients. Therefore, pharmacologic agents that strengthen the collateral vascular response to vasoconstrictors may contribute to a better hemostasis in patients with variceal bleeding. However, the clinical relevance of non-selective cyclooxygenase blockade is questionable due to the concern of renal decompensation: in cirrhotic patients, indomethacin for 7 consecutive days greatly impaired renal plasma flow, creatinine clearance and serum creatinine. Selective cyclooxygenase-2 inhibition seems promising since it does not significantly impair kidney function in cirrhotic rats with ascites. Recently, we have demonstrated with various nitric oxide synthase (NOS) inhibitors that constitutive rather than inducible NOS is involved in the vascular response of portal-systemic collaterals of portal hypertensive rats. Therefore, further survey with selective cyclooxygenase inhibitors might address the issue.

On the other hand, the degree of portal-systemic shunting as indicated by the slope of the flow-pressure curve was not significantly altered by chronic indomethacin treatment. Two possible origins of collateral vessels in portal hypertensive models have been proposed: passive dilatation of pre-existing venous channels and/or neovascularization. In terms of vascular hyperemia with dilatation encountered in portal hypertensive status, Fernandez et al had indicated that the reduction of mesenteric arterial blood flow in portal hypertensive rats was blunted to long-term indomethacin treatment (5 mg/kg/day, 7-day continuous infusion) as compared with acute treatment. At the same time, they also found an enhanced mesenteric vascular response to a non-selective NOS inhibitor, N(G)-nitro-L-arginine methyl ester. Since NO is another endothelium-derived vasodilator that contributes to the increased mesenteric arterial blood flow in portal hypertensive status, enhanced NO may compensate for the lack of vasodilatory prostaglandins in response to chronic indomethacin administration. Regarding new vessel formation, although indomethacin inhibited

**Discussion**

Indomethacin is a potent inhibitor of the cyclooxygenases in the synthesis of various prostaglandins and has been surveyed in various pathologic conditions. Many investigations find that the hyperdynamic circulation of portal hypertension can be attenuated by indomethacin. However, the systemic and portal hemodynamics did not reach remarkable changes in the present study. A similar finding has been reported with daily injection of indomethacin (4 mg/kg/body weight) for 2 days in rats after partial portal vein ligation. The actual reason for the contradictory results is not clear and might be due to different experimental designs. However, vascular homeostasis maintained by endogenous vasoactive substances might be considered.

For instance, unaltered blood pressure due to reciprocal interrelationship between nitric oxide (NO) and prostaglandins has been observed and indomethacin enhances interleukin-1β-induced nitrite release.

In a previous study, indomethacin incubation significantly potentiated the response of portal-systemic collaterals to AVP in portal hypertensive rats. The enhanced response to AVP by indomethacin suggested the role of prostaglandin in the regulation of the portal-systemic collateral circulation. We also found in the current study that long-term use of indomethacin improved the collateral vascular responsiveness to AVP in BDL cirrhotic rats. Gastroesophageal varices are the most prominent portal-systemic collaterals in cirrhotic patients. Therefore, pharmacologic agents that strengthen the collateral vascular response to vasoconstrictors may contribute to a better hemostasis in patients with variceal bleeding. However, the clinical relevance of non-selective cyclooxygenase blockade is questionable due to the concern of renal decompensation: in cirrhotic patients, indomethacin for 7 consecutive days greatly impaired renal plasma flow, creatinine clearance and serum creatinine. Selective cyclooxygenase-2 inhibition seems promising since it does not significantly impair kidney function in cirrhotic rats with ascites. Recently, we have demonstrated with various nitric oxide synthase (NOS) inhibitors that constitutive rather than inducible NOS is involved in the vascular response of portal-systemic collaterals of portal hypertensive rats. Therefore, further survey with selective cyclooxygenase inhibitors might address the issue.

On the other hand, the degree of portal-systemic shunting as indicated by the slope of the flow-pressure curve was not significantly altered by chronic indomethacin treatment. Two possible origins of collateral vessels in portal hypertensive models have been proposed: passive dilatation of pre-existing venous channels and/or neovascularization. In terms of vascular hyperemia with dilatation encountered in portal hypertensive status, Fernandez et al had indicated that the reduction of mesenteric arterial blood flow in portal hypertensive rats was blunted to long-term indomethacin treatment (5 mg/kg/day, 7-day continuous infusion) as compared with acute treatment. At the same time, they also found an enhanced mesenteric vascular response to a non-selective NOS inhibitor, N(G)-nitro-L-arginine methyl ester. Since NO is another endothelium-derived vasodilator that contributes to the increased mesenteric arterial blood flow in portal hypertensive status, enhanced NO may compensate for the lack of vasodilatory prostaglandins in response to chronic indomethacin administration. Regarding new vessel formation, although indomethacin inhibited

---

**Figure 1.** Concentration-response curves to arginine vasopressin (AVP) in the portal-systemic collateral vascular beds of indomethacin- and distilled-water (control)-treated bile duct-ligated rats, expressed as absolute increase over baseline value.

**Figure 2.** Flow-pressure relationship in perfused portal-systemic collateral vascular beds of indomethacin- and distilled-water (control)-treated bile duct-ligated rats.
neovascularization of rabbit cornea, a similar effect was not found in portal-systemic collaterals. Since NO has also been found to participate in the process of neovascularization in portal hypertensive rats, the counter-regulation by NO under the setting of prostaglandin synthesis inhibition should be considered, which deserves further evaluation.

Long-term indomethacin administration did not elicit changes in the EC50 values in the portal-systemic collaterals of BDL rats in the current study, similar to our previous finding in acute study. Apart from the receptor-mediated vasoconstricting effect, the non-receptor-mediated vasoconstriction on collaterals induced by potassium chloride was not changed. The lack of effect on baseline perfusion pressure for indomethacin observed in the present study has also been reported in normal rat liver perfusion.

In conclusion, chronic indomethacin administration in BDL rats enhances the portal-systemic collateral vascular responsiveness to AVP without modification of the severity of portal-systemic shunting.

Acknowledgments

The authors gratefully acknowledge Yun-Ni Hsieh for her excellent technical assistance. This work was supported by grants from the National Science Council (NSC 93-2314-B-075-060) and Taipei Veterans General Hospital (VGH-94-222), Taiwan.

References


