

Inhibition of Nitric Oxide Synthase in Hyperdynamic Circulation of Rats with Early or Late Cirrhosis Secondary to Common Bile Duct Ligation

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Abstract

Background/Aims: Preventing internal hemorrhage extends the lifespan of rats with chronic bile duct ligation (CBDL), a common animal model of portal hypertension. We investigated hemodynamics during the early and late stages of cirrhosis caused by CBDL. We also evaluated the hemodynamic influence of NO, which is the chief vasodilator in hyperdynamic syndrome, by administration of an NO synthase inhibitor (N^G-nitro-L-arginine methyl ester: L-NAME; 10 mg/kg).

Animals/Methods: In 24 Sprague-Dawley rats (9 sham rats and 15 CBDL rats), hemodynamics were assessed under conscious and unrestrained conditions 4 and 8 weeks after surgery. Before and 30 minutes after L-NAME administration, the cardiac index (CI) and regional blood flow were measured with the reference sample method using ¹⁴¹Ce- and ¹¹³Sn-microspheres (15 µm in diameter).

Results: A hyperdynamic systemic circulation and splanchnic hyperemia were observed after CBDL, and these changes increased with the progression of cirrhosis. L-NAME significantly diminished the hyperdynamic circulation and also reduced splanchnic hyperemia. In 4-week CBDL rats, a low hemoglobin concentration made an important contribution to the hyperdynamic circulation, and the portal collateral system collapsed when inflow to the portal territory was reduced by L-NAME treatment. In 8-week CBDL rats, systemic hemodynamics were closely linked to both the splanchnic circulation and the renal circulation before and after L-NAME administration, apart from hepatic artery blood flow.

Conclusion: The distinctive hemodynamic changes of portal hypertension were found in 8-week CBDL rats. Thus, 8-week CBDL rats may be a better animal model of human portal hypertension than 4-week CBDL rats.

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Key words: portal hypertension, nitric oxide, bile duct proliferation, splanchnic hyperemia, hyperdynamic circulation

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Introduction

Common bile duct ligation (CBDL) is widely used to create animal models of portal hypertension due to biliary cirrhosis¹⁻³. The lifespan of rats with CBDL is limited to 6 weeks because of fatal hemorrhage into various organs or body cavities due to impaired hemostasis⁴. Generally, portal hypertension with hyperdynamic syndrome develops gradually over several months or years in patients with cirrhosis. Accordingly, portal hypertension seen in animals 4 weeks after CBDL may represent the acute or subacute stage of this disorder and may precede the period when hemodynamic impairment reaches a steady state and manifests as hyperdynamic syndrome⁵. Thus, a longer interval after CBDL may be more appropriate when animal models are used to investigate human portal hypertension. We have previously demonstrated that preventing fatal hemorrhage can extend the lifespan of CBDL rats beyond 8 weeks⁴; therefore, studying hemodynamics in the chronic stage of biliary cirrhosis would become possible. To our knowledge, however, hemodynamics beyond 8 weeks after CBDL have not been investigated, except for in our previous study⁶.

The primary importance of vasodilatation in initiating hyperdynamic syndrome in patients with portal hypertension has been recognized by many investigators. As a rule, a single agent cannot explain a whole syndrome, but it has become evident that nitric oxide (NO) is the most important molecule responsible for the vasodilatation and multiple organ dysfunction that characterize hyperdynamic syndrome⁷⁻⁹. In the present study, we compared hemodynamics between CBDL rats with early and late cirrhosis and investigated the contribution of NO to hyperdynamic circulation by inhibiting NO synthase (NOS) in early and late CBDL rats. Finally, the validity of late CBDL rats as an animal model of portal hypertension was examined.

Materials and Methods

Creation of CBDL Rats

Twenty-four male Sprague-Dawley rats weighing 200 to 250 g were used. The protocol of this study

was reviewed and approved by the Ethics Committee for Animal Experiments of Nippon Medical School. Bile duct ligation was performed in 15 rats under sterile conditions. Anesthesia was induced with ether, followed by intraperitoneal pentobarbital sodium (50 mg/kg). The common bile duct was carefully exposed and double-ligated with silk threads, after which a 2-mm segment was excised between the ligatures to prevent regeneration. Sham surgery was performed in another 9 rats by mobilization of the common bile duct without ligation or excision. Hemodynamic variables measured in intact sham-operated rats were used as standard values in this study. Animals were examined 4 or 8 weeks after CBDL or 4 weeks after sham surgery. The CBDL rats were given menatetrenone (vitamin K: 2.5 mg/kg) once a week from the beginning of the 3rd week after surgery to the beginning of the last study week. After the experiments had been completed, the animals were killed by an overdose of intravenous pentobarbital sodium, after which autopsy was performed to macroscopically confirm the development of cirrhosis and portal hypertension. In addition, histological examination of the liver was performed in several other animals that did not belong to either of the study groups. The laboratory profile of our CBDL rats is shown in **Table 1**⁶.

Experimental Preparation

Catheters for hemodynamic measurements were inserted on the day before the experiments with the same anesthesia described above. All catheters were tunneled subcutaneously and exteriorized at the back of the neck. The animals were allowed to recover from the surgical procedure for 24 hours with overnight fasting but free access to water. All studies were performed on conscious and unrestrained animals. Each animal was left alone for at least 30 minutes after installation of the experimental apparatus until a stable physiological condition was reached (i.e., minimal fluctuation of arterial pressure with a stable heart rate or minimal body motion or both). Hemodynamic measurements were performed in a quiet and air-conditioned room maintained at 24°C.

NOS Inhibitor

N^G-nitro-L-arginine methyl ester hydrochloride (L-

Table 1 Body weight and laboratory data of the three groups⁶

	Sham rats (n=8)	4-wk CBDL rats (n=8)	8-wk CBDL rats (n=9)	p value
Body weight (g)	341 ± 15	328 ± 26 [‡]	370 ± 34 [§]	p=0.011
Hemoglobin (g/dL)	12.5 ± 0.4	11.6 ± 1.9 [‡]	13.6 ± 0.7	p=0.007
Total bilirubin (mg/dL)	0.0 ± 0.0 [†]	8.5 ± 1.2 [‡]	6.3 ± 1.4 [§]	p=0.000
Direct bilirubin (mg/dL)	0.0 ± 0.0 [†]	7.3 ± 1.0 [‡]	4.9 ± 1.0 [§]	p=0.000
ALT (IU/L)	40.8 ± 2.3 [†]	205.3 ± 77.7	212.9 ± 62.0 [§]	p=0.000
ALP (IU/L)	485.6 ± 57.0 [†]	1,496.3 ± 386.1 [‡]	1,819.6 ± 372.5 [§]	p=0.000
Albumin (g/dL)	4.0 ± 0.1 [†]	3.7 ± 0.2 [‡]	3.1 ± 0.3 [§]	p=0.000
NOx (μMol/L)	12.9 ± 1.2 ^{P1}	17.5 ± 3.8	19.4 ± 6.5 [§]	p=0.009
Endothelin-1 (μMol/L)	1.76 ± 0.65 [†]	9.45 ± 1.48 [‡]	14.09 ± 2.49 [§]	p=0.000

Values are the mean ± SD. ALT, alanine aminotransferase; ALP, alkaline phosphatase; NOx, nitrate + nitrite; p values for one-way ANOVA with post hoc comparison by Fisher's PLSD test.

[†]p<0.05 vs. 4-wk CBDL, [‡]p<0.05 vs. 8-wk CBDL, [§]p<0.05 vs. Sham. ^{P1}p=0.052 vs. 4-wk CBDL

NAME, Wako Pure Chemical Industries Ltd., Tokyo), a nonspecific NOS inhibitor, was dissolved in 0.25 mL of physiological saline and administered intravenously at a dose (10 mg/kg). This dose of L-NAME corresponded to that having 80% of its maximum effect on arterial pressure, based on the dose-response curve for arterial pressure in normal rats. Systemic and splanchnic hemodynamics were assessed before and 30 minutes after administration of L-NAME.

Measurement of Systemic and Splanchnic Hemodynamics

Hemodynamic measurements were performed before and 30 minutes after L-NAME administration. Arterial pressure and portal pressure (PP) were directly measured via catheters placed in the right femoral artery and at the junction of the superior mesenteric and splenic veins, respectively. The heart rate was determined by counting the arterial pressure waves. The cardiac index (CI) was measured before and after L-NAME administration with the reference sample method using ¹⁴¹Ce- and ¹¹³Sn-microspheres with a diameter of 15 μm (NENTM Life Science Products, Inc., Boston, MA, USA). In brief, 100 × 10⁴ radiolabeled microspheres suspended in 0.8 mL of saline containing 10% Ficoll-70 were gradually injected over 60 seconds through a silastic catheter in the left ventricle, and simultaneously a 0.8-mL reference blood sample was withdrawn (at a constant rate over 60 seconds) from a catheter in the abdominal aorta using a medical pump (Model 2400-003, Harvard Apparatus, Holliston, MA, USA).

The radioactivity of the microspheres (before injection) and that of the reference blood sample were counted with a gamma-counter (Aloka 505, Aloca Ltd., Tokyo, Japan) at a setting of 116–174 keV for ¹⁴¹Ce and 314–470 keV for ¹¹³Sn, and the results were corrected using a ¹⁴¹Ce or ¹¹³Sn standard. Data were separated with spectral deconvolution. Adequate mixing of microspheres was ensured by confirming a difference in radioactivity of <10% between the left and right kidneys.

Calculations:

CI=[injected radioactivity (cpm)/reference sample radioactivity (cpm)] × [100/BW (g)] × 0.8 (mL/min) (mL/min/100 g BW).

Total systemic vascular resistance (TSVR)=
MAP × 80/CI (dynes · sec · cm⁻⁵/100 g BW).

Organ blood flow=[organ radioactivity (cpm)/injected radioactivity (cpm)] × CI (mL/min/100 g BW).

Portal territory blood flow (PTBF) was defined as the sum of the blood flow through the stomach, intestine, colon, pancreas, spleen, and mesentery.

Portal territory vascular resistance (PTVR)=
(MAP-PP) × 80/PTBF (dynes · sec · cm⁻⁵/100 g BW)

Hepatic artery vascular resistance (HAVR)=
MAP × 80/HABF (dynes · sec · cm⁻⁵/100 g BW)

Renal artery vascular resistance (RAVR)=
MAP × 80/RABF (dynes · sec · cm⁻⁵/100 g BW)

Hepatocollateral vascular resistance (HCVR)=
PP × 80/PTBF (dynes · sec · cm⁻⁵/100 g BW),

where BW is body weight, MAP is mean arterial pressure, HABF is hepatic artery blood flow, HAVR is hepatic artery vascular resistance, PP is portal

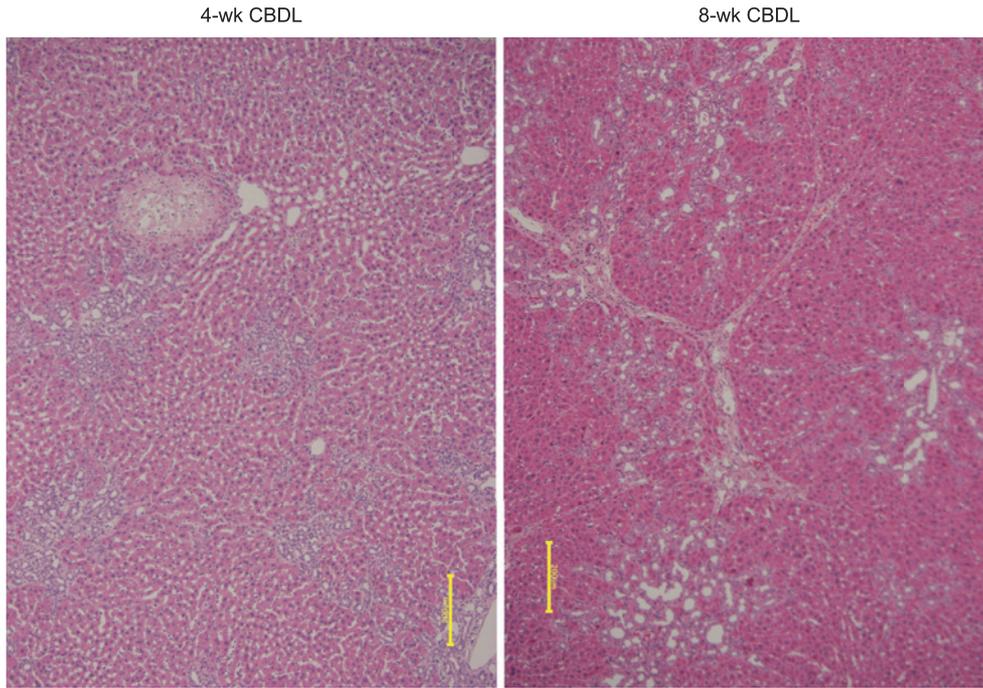


Fig. 1 Liver histology in a 4-week CBDL rat and an 8-week CBDL rat. Photomicrographs of standard hematoxylin and eosin-stained sections obtained with an Olympus AX-80/DP-50 digital camera. (yellow bars=200 μ m)

pressure, RABF is renal artery blood flow, and RAVR is renal artery vascular resistance.

Statistical Analysis

Statistical analysis of differences between groups was performed with one-way analysis of variance, and post-hoc comparisons were performed with Fisher's protected least significant difference test and the paired or unpaired *t*-test as appropriate. Linear correlation analysis was performed with Statistica software program (StatSoft™ Inc., Tulsa, OK, USA). Results are expressed as the means \pm standard deviation (SD). Statistical significance was defined at $p < 0.05$.

Results

Histological findings of the liver in 4-week or 8-week CBDL rats: Histological examination of hematoxylin/eosin-stained liver specimens from an 8-week CBDL rat showed bridging fibrosis linking the portal tracts and excessive bile duct proliferation (**Fig. 1**). In a 4-week CBDL rat, bile duct proliferation was similarly seen, and bile infarcts were occasionally observed, but bridging fibrosis was

immature.

Baseline hemodynamics: In the baseline state, CBDL rats had a hyperdynamic systemic circulation with decreased TSVR and hypotension that showed progression along with the exacerbation of liver damage (**Table 2**). Arterial hypotension, portal hypertension, an increase of HABF, and renal hyperemia in 8-week CBDL rats were more severe than in 4-week CBDL rats.

Effect of NOS inhibition on systemic and splanchnic hemodynamics: Administration of L-NAME attenuated the hyperdynamic systemic circulation in both 4-week and 8-week CBDL rats (**Table 2**). In both groups of CBDL rats, the CI decreased to a similar value as that in sham-operated rats, whereas MAP increased above that of sham-operated rats. Elevation of PP, the increase in PTBF, and the decline in PTVR were minimized by L-NAME treatment in both groups of CBDL rats. In 4-week CBDL rats, the percent decrease in PP due to L-NAME treatment was particularly marked, as was the concomitant reduction of percent HCVR (**Fig. 2**). In 8-week CBDL rats, however, the percent decrease in PP was mild, and HCVR was maintained. The HABF increased (with a fall in HAVR) with

Table 2 Hemodynamics of Sham rats and hemodynamics before and after L-NAME administration in 4-wk CBDL rats and 8-wk CBDL rats

	Sham rats (n=9)	4-wk CBDL rats (n=7)		p value	8-wk CBDL rats (n=8)		p value
		Before	After		Before	After	
Hemoglobin [#]	12.2 ± 0.5 [†]	10.6 ± 0.9 [‡]			13.0 ± 1.9		0.003
Systemic hemodynamics							
HR (bpm)	341 ± 16 [†]	397 ± 34	354 ± 26 [§]	0.022	376 ± 42 [§]	324 ± 34	0.003
MAP (mmHg)	107.4 ± 8.2 ^{P1}	100.7 ± 6.1 [‡]	128.6 ± 10.7	0.000	92.5 ± 7.6 [§]	124.4 ± 8.2	0.000
CI [¶]	27.4 ± 3.4 [†]	37.21 ± 3.82	23.78 ± 3.08 [§]	0.000	39.20 ± 6.56 [§]	28.81 ± 6.11	0.000
TSVR [*]	318.8 ± 53.5 [†]	218.0 ± 24.9	438.9 ± 71.6 [§]	0.000	194.2 ± 40.4 [§]	360.4 ± 82.8	0.000
Splanchnic circulation							
PP (mmHg)	5.0 ± 0.7 [†]	14.9 ± 1.2 [‡]	10.2 ± 1.3 [§]	0.000	16.5 ± 1.1 [§]	14.3 ± 1.8	0.001
PTBF [¶]	5.8 ± 1.0 [†]	7.6 ± 0.9	6.1 ± 1.0	0.003	8.3 ± 1.5 [§]	6.8 ± 1.5	0.002
PTVR [*]	1,469 ± 317 [†]	911 ± 132	1,586 ± 319	0.000	757 ± 158 [§]	1,361 ± 309	0.000
HABF [¶]	0.45 ± 0.29 [†]	1.44 ± 0.53 [‡]	1.41 ± 0.50 [§]	0.798	2.64 ± 0.96 [§]	2.67 ± 1.25	0.953
HAVR [*]	28,303 ± 20,036 [†]	6,721 ± 3,995	8,945 ± 5,761 ^{P4}	0.042	3,117 ± 1,078 [§]	4,427 ± 1,797	0.003
HCVR [*]	71.7 ± 17.4 [†]	158.9 ± 25.6	136.2 ± 25.3 [§]	0.033	163.2 ± 24.5 [§]	173.4 ± 26.3	0.461
Renal circulation							
RABF [¶]	4.17 ± 0.84 [†]	5.86 ± 0.97 ^{P2}	3.69 ± 0.76	0.000	7.31 ± 2.11 [§]	4.68 ± 1.32	0.000
RAVR [*]	2,126 ± 394 [†]	1,404 ± 224 ^{P3}	2,861 ± 517	0.000	1,090 ± 343 [§]	2,275 ± 624	0.000

Values are expressed as the mean ± SD. HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; TSVR, total systemic vascular resistance; PP, portal pressure; PTBF, portal tributary blood flow; PTVR, portal tributary vascular resistance; HABF, hepatic arterial blood flow; HAVR, hepatic arterial vascular resistance; RABF, renal arterial blood flow; RAVR, renal arterial vascular resistance. [#]g/dL; ^{*}dynes.sec.cm⁻⁵/100 g body weight; [¶]mL/min/100 g body weight. [†]p<0.05 vs. 4-wk CBDL rats (before); [‡]p<0.05 vs. 8-wk CBDL rats (before); [§]p<0.05 vs. Sham rats ^{P1}p=0.088 vs. 8-wk CBDL (before); ^{P1}p=0.087 vs. 4-wk CBDL (before); ^{P2}p=0.062 vs. 8-wk CBDL rats (before); ^{P3}p=0.085 vs. 8-wk CBDL (before) [§]p<0.05 (unpaired *t*-test) vs. 8-wk CBDL (after); ^{P4}p=0.055 (unpaired *t*-test) vs. 8-wk CBDL (after). Comparison of hemodynamics in Sham rats and hemodynamics before L-NAME administration in CBDL rats was done by one-way ANOVA with post hoc comparison using Fisher's PLSD test. P values indicate the significance of difference between before and after L-NAME administration (paired *t*-test).

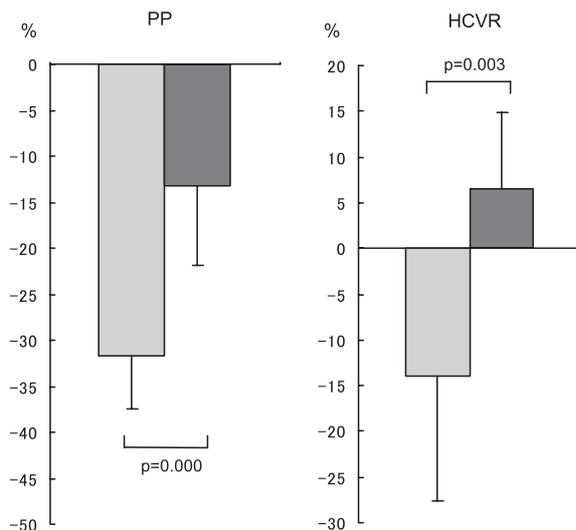


Fig. 2 Percent change in portal pressure (PP) and percent change in hepatocollateral vascular resistance (HCVR) in 4-week and 8-week CBDL rats.

Gray bar indicates 4-week CBDL rats (n=7), and dark gray bar indicates 8-week CBDL rats (n=8).

liver damage but was not affected (with a slight increase in HAVR) by L-NAME treatment in either 4-week or 8-week CBDL rats (**Fig. 3A**). On the other hand, renal hyperemia increased with liver damage but was improved by administration of L-NAME (**Fig. 3B**). The percent change in CI with L-NAME administration was greater in 4-week CBDL rats (-35.8 ± 7.9%) than in 8-week CBDL rats (-26.6 ± 8.8%, p=0.055). The percent change in CI was significantly correlated with the hemoglobin concentration (**Fig. 4**), which was significantly lower in 4-week CBDL rats than in sham-operated rats or 8-week CBDL rats (**Table 2**).

Systemic hemodynamics and splanchnic circulation: In 8-week CBDL rats, there was a significant (or sometimes weak) positive correlation between CI and PTBF (**Fig. 5**) or PP (**Fig. 6**) before and after L-NAME administration. There was also a significant positive correlation between PP and

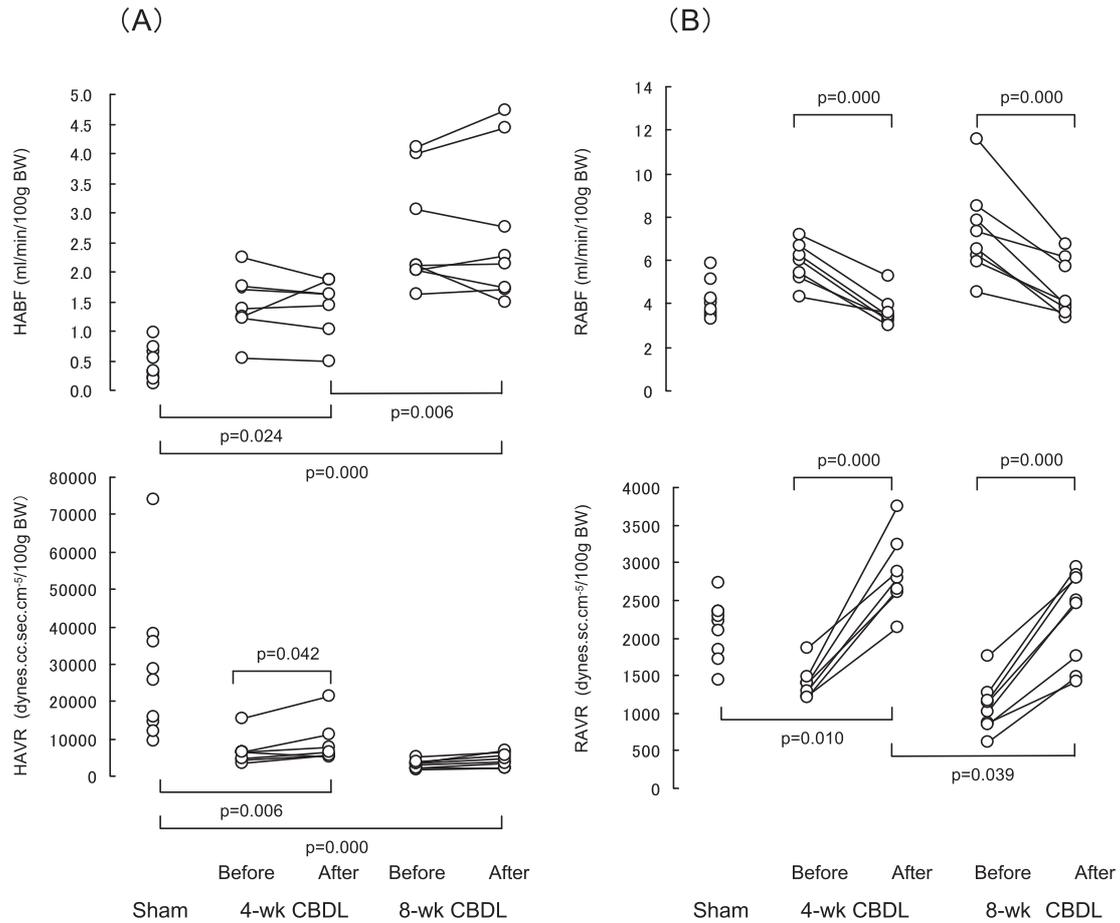


Fig. 3 Effect of nitric oxide synthase (NOS) inhibition by intravenous L-NAME (10 mg/kg) on (A) hepatic artery variables and (B) renal artery variables in individual 4-week CBDL rats (n=7) and 8-week CBDL rats (n=8), as well as baseline values for sham-operated rats (n=9). HABF, hepatic artery blood flow; HAVR, hepatic artery vascular resistance; RABF, renal artery blood flow; RAVR, renal artery vascular resistance.

RABF before and after L-NAME administration in the 8-week CBDL rats (**Fig. 7**). When the correlations between variables were investigated in 4-week CBDL rats, we could not find any significant association between systemic hemodynamics and splanchnic or renal hemodynamics, apart from a positive correlation of CI with RABF before L-NAME administration and a negative correlation of PP with HABF after L-NAME administration ($r=0.779$, $n=7$, $p=0.039$ and $r=-0.790$, $n=7$, $p=0.035$, respectively).

Discussion

As expected, 8-week CBDL rats showed more histologically advanced liver cirrhosis than did 4-week CBDL rats. This finding does not contradict

the laboratory results of our previous study⁶, which demonstrated severe impairment of liver synthetic function (decline of serum albumin) in 8-week CBDL rats. An increase in the urinary excretion of direct (water soluble) bilirubin, probably due to an increase in RABF, might account for the decrease of total bilirubin in 8-week CBDL rats and does not suggest improvement of their condition. Along with the progression of liver damage from 4 weeks to 8 weeks after CBDL, both the hyperdynamic systemic circulation and splanchnic hyperemia became more severe. In particular, the decrease in MAP, elevation of PP, and increase in HABF or RABF were greater in 8-week CBDL rats than in 4-week CBDL rats.

Many investigators have indicated that overproduction of NO plays a major role in starting the vasodilatation that causes hyperdynamic

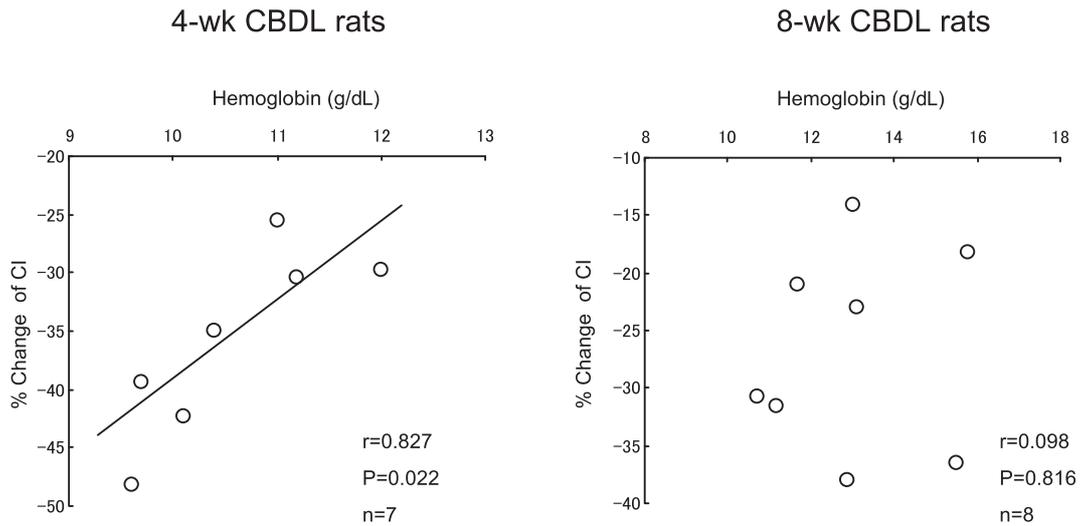


Fig. 4 Correlation between the percent change in the cardiac index (% change of CI) and the hemoglobin concentration in 4-week CBDL rats (n=7) and 8-week CBDL rats (n=8).

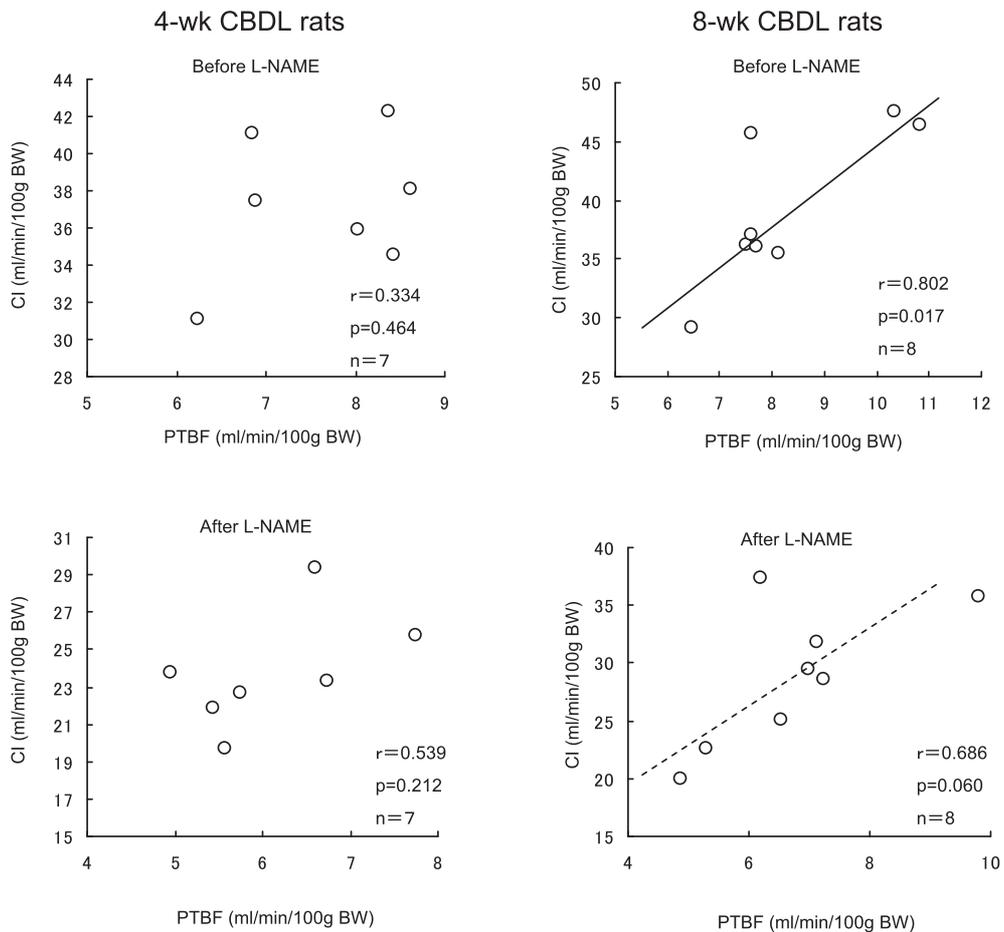


Fig. 5 Correlations between the cardiac index (CI) and portal tributary blood flow (PTBF) before and after intravenous L-NAME treatment (10 mg/kg) in 4-week CBDL rats (n=7) and 8-week CBDL rats (n=8).

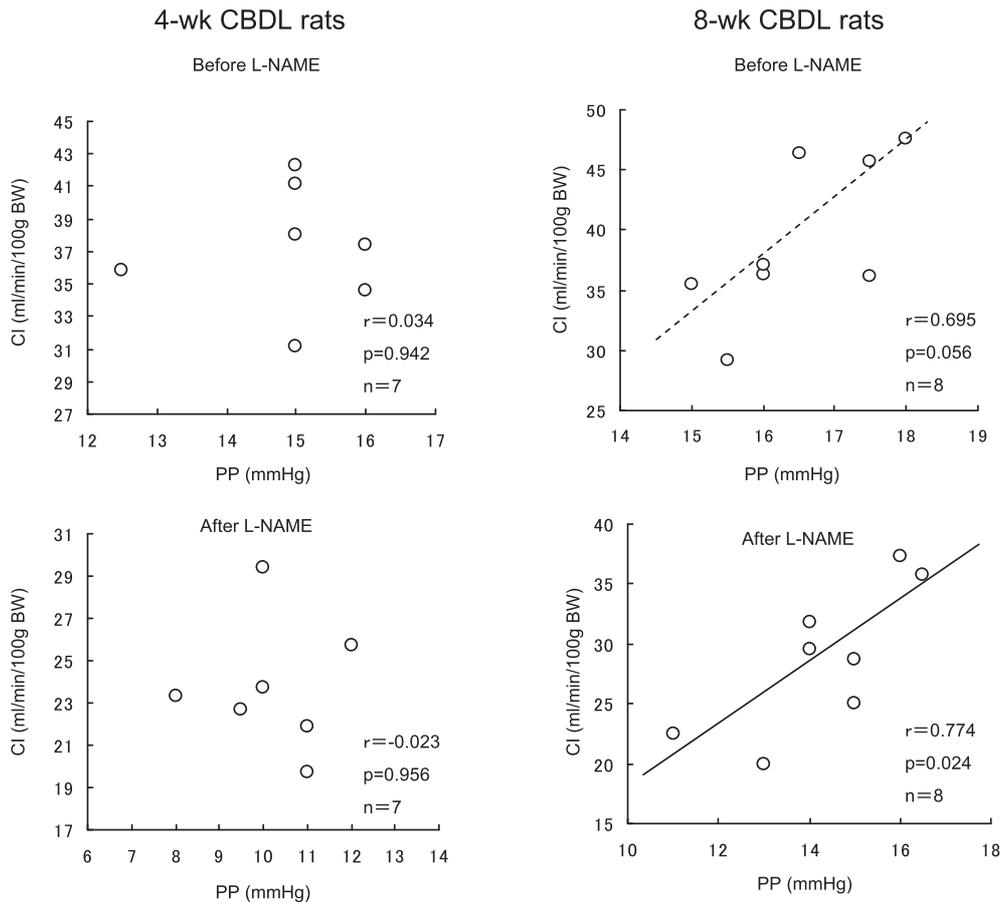


Fig. 6 Correlations between the cardiac index (CI) and portal pressure (PP) before and after intravenous L-NAME treatment (10 mg/kg) in 4-week CBDL rats (n=7) and 8-week CBDL rats (n=8).

syndrome associated with portal hypertension^{10,11}. Certainly, we found that inhibition of NOS markedly attenuated the systemic hyperdynamic circulation and alleviated splanchnic hyperemia along with an increase in vascular resistance in both groups of CBDL rats. However, there were some differences in the hemodynamic response to NOS inhibition between 4-week and 8-week CBDL rats. The rise of TSVR and concomitant decrease of CI after inhibition of NOS in 4-week CBDL rats were greater than in 8-week CBDL rats. The anemia seen in 4-week CBDL rats might have prolonged the half-life of NO due to less scavenging from the peripheral circulation¹², which might then have reduced vascular tone even further and augmented the hyperdynamic changes. The significant positive correlation between the hemoglobin concentration and the percent change in CI after L-NAME administration in 4-week CBDL rats supports this assumption. In 8-week CBDL rats, however, we could not find any contribution of hemoglobin to

hemodynamic derangement. It is difficult to explain the anemia in 4-week CBDL rats, but various events related to acute liver damage might affect hematopoiesis or reduce the lifespan of erythrocytes. Beyond 8 weeks, the pathological changes probably reach a chronic state, and anemia recovers.

In 4-week CBDL rats, PP decreased markedly along with a striking decline of HCVR in response to the reduction in PTBF by administration of L-NAME. It is possible that the portal venous system was not fully adapted to the rise of portal pressure in 4-week CBDL rats, and their newly developed portal collateral vessels might also have immature contractile function. Therefore, the sudden reduction in PTBF after L-NAME administration may have resulted in collapse of the portal venous system in 4-week CBDL rats. In 8-week CBDL rats, however, there was only a moderate decrease in PP with little change in HCVR after L-NAME treatment. Persistent elevation of PP leads to vasculopathy that affects the contractility of the portal vein¹³. In 8-week

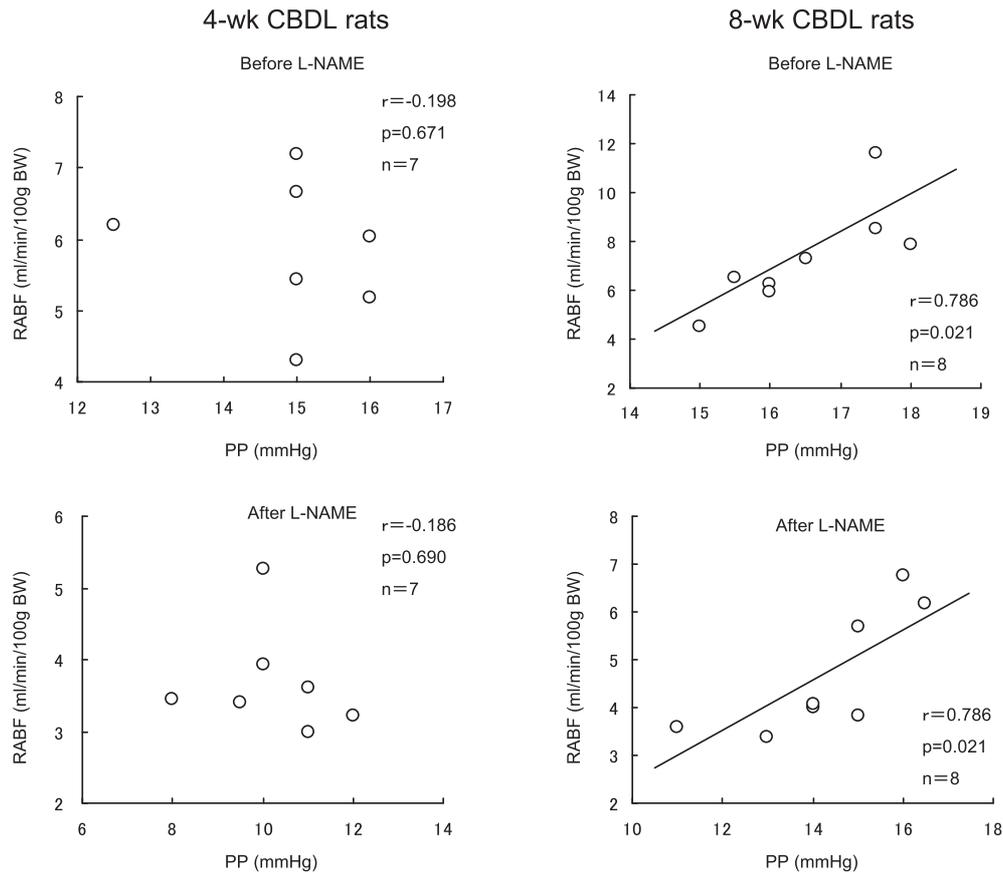


Fig. 7 Correlation between renal artery blood flow (RABF) and portal pressure (PP) before and after intravenous L-NAME treatment (10 mg/kg) in 4-week CBDL rats (n=7) and 8-week CBDL rats (n=8).

CBDL rats, it appears that the portal venous system and its collateral vessels were well developed, and thus responded adequately to the acute reduction of PTBF after L-NAME treatment.

In both groups of CBDL rats, HABF and RABF increased similarly liver damage progressed. However, the response to NOS inhibition differed between HABF and RABF, with the former not being affected and the latter decreasing after treatment. A compensatory increase in HABF, known as the hepatic arterial buffer response (HABR), may maintain the blood supply to the liver when hepatopetal portal blood flow is reduced owing to cirrhotic portal hypertension^{14,15}. ADP plays the main role in activating the HABR, and NO also participates to some extent¹⁶. However, NO appears to make little contribution to modulating HABF, because inhibition of NOS led to a slight increase in HAVR but did not reduce HABF in our animal model. It may be that the increase in HABF is not only dependent on HABR, but is also influenced by

other mechanism(s). Because bile duct proliferation due to biliary obstruction is accompanied by expansion of the peribiliary plexus, which arises from the hepatic artery, it is more rational to consider that exaggerated bile duct proliferation in CBDL rats causes a passive increase in HABF¹⁷.

Unlike patients with cirrhosis, in whom RABF decreases as liver disease progresses^{18,19}, experimental models of portal hypertension show renal hyperemia²⁰⁻²². An increase in NO production in the glomeruli may cause renal hyperemia in experimental portal hypertension²³. In contrast to the response of HABF, the increase in RABF was completely abolished by administration of L-NAME in both groups of CBDL rats, because RABF declined to a similar level as that in sham-operated rats. This result indicates that NO is an important modulator of renal hyperemia in CBDL rats.

In summary, aggravation of the systemic hyperdynamic circulation and splanchnic hyperemia were observed in the late stage (8 weeks) after

CBDL. The portal venous system, including collateral vessels, was functionally well developed. Furthermore, there was a close correlation between the systemic and splanchnic circulation in the late stage. In contrast, in rats with early CBDL, systemic hemodynamics were strongly influenced by anemia and the portal collateral system was immature. Although HABF and RABF progressed with cirrhosis, the HABF was not influenced by inhibition of NOS. As for the RABF, its increase was completely prevented by L-NAME in both the early and late stages of CBDL. Based on these results, we conclude that rats with late-stage CBDL have steady-state hemodynamic abnormalities related to portal hypertension. It should be remembered, however, that cirrhosis due to CBDL is associated with marked bile duct proliferation unlike the common causes of cirrhosis in humans.

References

1. Lee SS, Girod C, Braillon A, Hadengue A, Lebrec D: Hemodynamic characterization of chronic bile duct-ligated rats: effect of pentobarbital sodium. *Am J Physiol* 1986; 251: G176-180.
2. Blanchet L, Lebrec D: Changes in splanchnic blood flow in portal hypertensive rats. *Eur J Clin Invest* 1982; 12: 327-330.
3. Zhang XJ, Katsuta Y, Akimoto T, Ohsuga M, Aramaki T, Takano T: Intrapulmonary vascular dilatation and nitric oxide in hypoxemic rats with chronic bile duct ligation. *J Hepatol* 2003; 39: 724-726.
4. Akimoto T, Hayashi N, Adachi M, et al.: Valiability and plasma vitamin K levels in the common bile duct-ligated rats. *Exp Anim* 2005; 54: 155-161.
5. Newby DE, Hayes PC: Hyperdynamic circulation in liver cirrhosis: not peripheral vasodilatation but splanchnic steal. *QJM* 2002; 95: 827-830.
6. Katsuta Y, Zhang XJ, Ohsuga M, et al.: Hemodynamic features of advanced cirrhosis due to chronic bile duct ligation. *J Nippon Med Sch* 2005; 72: 217-225.
7. Sogni P, Sabry S, Moreau R, Gadano A, Lebrec D, Dinh-Xuan AT: Hyporeactivity of mesenteric resistance arteries in portal hypertensive rats. *J Hepatol* 1996; 24: 487-490.
8. Lee FY, Colombato LA, Albillos A, Groszmann RJ: Administration of N omega-nitro-L-arginine ameliorates portal-systemic shunting in portal-hypertensive rats. *Gastroenterology* 1993; 105: 1464-1470.
9. Groszmann RJ: Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. *Hepatology* 1994; 20: 1359-1363.
10. Sieber CC, Groszmann RJ: Nitric oxide mediates hyporeactivity to vasopressors in mesenteric vessels of portal hypertensive rats. *Gastroenterology* 1992; 103: 235-239.
11. Pizcueta MP, Piqué JM, Bosch J, Whittle BJ, Moncada S: Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension. *Br J Pharmacol* 1992; 105: 184-190.
12. Casadevall M, Pique JM, Cirera I, et al.: Increased blood hemoglobin attenuates splanchnic vasodilation in portal-hypertensive rats by nitric oxide inactivation. *Gastroenterology* 1996; 110: 1156-1165.
13. Li T, Yang Z: Research progress of vasculopathy in portal hypertension. *World J Gastroenterol* 2005; 11: 6079-6084.
14. Lauth WW: Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am J Physiol* 1985; 249 (5 Pt 1): G549-556.
15. Richter S, Mücke I, Menger MD, Vollmar B: Impact of intrinsic blood flow regulation in cirrhosis: maintenance of hepatic arterial buffer response. *Am J Physiol Gastrointest Liver Physiol* 2000; 279: G454-462.
16. Zipprich A, Mehal WZ, Ripoll C, Groszmann RJ: A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatory response in the CCl₄-cirrhotic liver. *Liver Int* 2010; 30: 988-994.
17. Masyuk TV, Ritman EL, LaRusso NF: Hepatic artery and portal vein remodeling in rat liver: vascular response to selective cholangiocyte proliferation. *Am J Pathol* 2003; 162: 1175-1182.
18. Arroyo V, Ginès P, Gerbes AL, et al.: Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. *International Ascites Club. Hepatology* 1996; 23: 164-176.
19. Ring-Larsen H: Renal blood flow in cirrhosis: relation to systemic and portal haemodynamics and liver function. *Scand J Clin Laboratory Invest* 1977; 37: 635-642.
20. Vallance P, Moncada S: Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; 337: 776-778.
21. Porst M, Hartner A, Krause H, Hilgers KF, Veelken R: Inducible nitric oxide synthase and glomerular hemodynamics in rats with liver cirrhosis. *Am J Physiol Renal Physiol* 2001; 281: F293-299.
22. García-Estañ J, Atucha NM, Sabio JM, Vargas F, Quesada T, Romero JC: Increased endothelium-dependent renal vasodilation in cirrhotic rats. *Am J Physiol* 1994; 267: R549-553.
23. Gaudio E, Onori P, Pannarale L, Alvaro D: Hepatic microcirculation and peribiliary plexus in experimental biliary cirrhosis: a morphological study. *Gastroenterology* 1996; 111: 1118-1124.

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