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RAPID COMMUNICATION

Local regulator adrenomedullin contributes to the circulatory disturbance in cirrhotic rats

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Abstract

AIM: To investigate whether adrenomedullin, a potent vasodilator peptide, plays a role in the circulatory disturbance in cirrhosis.

METHODS: Cirrhosis was induced in rats by weekly gavage of carbon tetrachloride. Hemodynamic studies were performed *in vivo* using radioactive microspheres and *in vitro* using isolated aortic rings. The adrenomedullin concentrations were measured by radioimmunoassay.

RESULTS: Acute administration of adrenomedullin to the control rats reduced the systemic arterial pressure along with an increase of serum levels of the stable metabolite of nitric oxide (NOx), in a dose-dependent manner. Chronic infusion of adrenomedullin reduced the vascular resistance and increased the blood flow in the systemic and splanchnic circulation. Intravenous administration of anti-adrenomedullin antibody did not affect any hemodynamic parameters in the cirrhotic rats, whereas this antibody ameliorated the blunted contractile response to phenylephrine, α-adrenergic receptor agonist, in the aortic rings of the cirrhotic rats. The adrenomedullin concentrations in the aorta were higher in the cirrhotic rats than in the controls, and correlated with the mean arterial pressure in the cirrhotic rats. Moreover, adrenomedullin blunted the contractile response to phenylephrine in both of the control aorta and cirrhotic aorta, but not in the presence of NG-nitro-L-arginine methyl ester, an NO synthase inhibitor.

CONCLUSION: Adrenomedullin overproduced in the vascular wall may contribute to the circulatory disturbance in cirrhosis as a local regulator of the vascular tonus rather than a circulating hormone.

Key words: Adrenomedullin; Liver cirrhosis; Vasodilation; Circulatory disturbance; Vascular tonus; Circulating hormone

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INTRODUCTION

Arterial hypotension, high cardiac output, low vascular resistance, and hyporeactivity to vasoconstrictors are hemodynamic features in human and experimental liver cirrhosis[1-3]. These circulatory disturbances may be attributed to arterial vasodilation that results from overproduction or reduced degradation of vasodilator substances[1-3]. Several circulating vasodilator peptides including substance P, calcitonin gene-related peptide, and glucagon are increased in the cirrhotic patients[4-6]. Moreover, many studies have suggested that the vasodilator substances produced in the vascular wall such as nitric oxide (NO) and carbon monoxide, an end product of the haeme oxygenase pathway, may be coordinately associated with arterial vasodilation in cirrhosis[7-9]. Therefore, the high levels of the circulating vasodilator peptides and the increased vascular production of vasodilators can contribute to the pathogenesis of arterial vasodilation leading to the circulatory disturbance in liver cirrhosis.

Adrenomedullin (AM) is the potent hypotensive peptide discovered in the human pheochromocytoma, and is considered to cause a potent vasodilation via synthesis of NO in the vascular endothelial cells as well as an increase of the intracellular adenosine 3',5'-cyclic monophosphate in the vascular smooth muscle cells[10-11]. AM is abundant in the adrenal medulla, but is widely distributed in the human and rat organs including the vascular tissue[12,13]. Several clinical studies have

demonstrated that the circulating AM levels are increased along with progression of the liver disease and correlate with the hemodynamic parameters in cirrhosis[14-17]. Moreover, the gene expression of AM in the vascular tissue was more enhanced in the cirrhotic rats than in the controls[18]. These findings raise the possibility that AM may be involved in the circulatory disturbance in cirrhosis. However, whether the increased circulating AM and/or the enhanced vascular production of AM plays a role in the circulatory disturbance in liver cirrhosis remains to be established. In this study, the role of the circulating AM in the hemodynamic derangement in cirrhosis was investigated in the presence of exogenous AM and/or anti-AM antibody using radioactive microspheres. Moreover, the AM concentrations in the aorta were evaluated in relationship to the systemic blood pressure, and the effect of AM on the vascular tonus was investigated in the presence of exogenous AM and/or anti-AM antibody using isolated aortic rings. This study aimed to investigate whether the increased circulating AM and/or the enhanced vascular production of AM plays a role in the circulatory disturbance in liver cirrhosis.

MATERIALS AND METHODS

Animal preparation

The experiments were performed on male Sprague-Dawley rats. Liver cirrhosis was induced by weekly intragastric administration of carbon tetrachloride[19]. The rats weighing about 150 g were given phenobarbital (35 mg/dL) in the drinking water. After 2 weeks when the rats were about 250 g, the initial dose (0.04 mL) of intragastric carbon tetrachloride was begun. Body weight was monitored and the dose of intragastric carbon tetrachloride was adjusted according to the change of body weight. After 8-10 doses of carbon tetrachloride, micronodular liver cirrhosis was induced in the most rats and the half of cirrhotic rats developed ascites. The control rats were treated with phenobarbital alone. All animals received humane care and all experiments were performed according to the guidelines of the Committee for the Care and Use of Laboratory Animals in Nara Medical University.

Hemodynamic studies

Hemodynamic studies were performed by the same operator to reduce the operator-dependent variability. Ketamine (100 mg/kg, i.m.) was used as an anesthetic drug, because it closely resembled the conscious state in terms of hemodynamics[20]. The left femoral artery, right jugular vein, femoral vein, and portal vein were cannulated with PE-50 catheters. The left ventricle was catheterized with another PE-50 tube. Each catheter used was filled with heparinized saline. The cardiac output and regional blood flow were measured using radioactive microspheres[21]. A reference sample was obtained from the femoral artery for 75 seconds at a rate of 1 mL/min using continuous withdrawal pump (CFV2100; Nihon Kohden, Tokyo, Japan). Approximately 60 000 microspheres labeled with ^{57}Co (15.5 ± 0.1 μm diameter, specific activity: 610 MBq/g; New England Nuclear, Boston, MA, USA) were injected into the left ventricle 15 seconds after the start of blood withdrawal. The cardiac output was calculated as follows: The cardiac output (mL/min) = injected radioactivity (cpm) × reference blood flow (mL/min) / reference blood radioactivity (cpm). The cardiac index was expressed per 100 g of body weight. The abdominal organs were cut into small pieces and placed in counting tubes. The radioactivity of each organ was determined with a gamma counter. For calculation of the regional blood flow, the injected radioactivity was replaced by radioactivity of each organ in the previous equation. The portal venous inflow was calculated as the sum of the blood flows to the stomach, spleen, small and large intestines, pancreas, and mesentery. The vascular resistance was calculated from the ratio between the perfusion pressure and the blood flow in each vascular territory. The hemodynamics were investigated as follows: 1) Control rats were infused with either vehicle (n = 6) or human AM (0.1, 0.3, 1.0 nmol/kg/min for 10 min, n = 6, respectively) via a femoral vein catheter. Another six control rats were given human AM at a dose of 0.3 nmol/kg/min for 10 min after an intravenous injection of anti-AM antibody (500 μg/kg). The mean arterial pressure and serum levels of NO× (NO_2^- + NO_3^-), a stable metabolite of NO, were determined before and after the infusion. 2) To compare the magnitude of depressor response of the exogenous AM, cirrhotic rats were also infused either vehicle (n = 6) or human AM (0.1, 0.3, 1.0 nmol/kg/min for 10 min, n = 6, respectively) and the mean arterial pressure was determined before and after the infusion. 3) Control rats were chronically infused with either vehicle (n = 8) or human AM (1.0 μg/h, n = 8) for 14 d using a mini-osmotic pump (alzet model 2002, Alza, CA, USA). The pumps were connected to the left jugular vein cannulated with PE-60 and placed in the subcutaneous tissue. The human AM dose was determined according to a previous study[22]. On d 14 of chronic infusion, hemodynamic studies were performed using radioactive microspheres. 4) Cirrhotic rats with ascites were repeatedly injected with either anti-AM antibody (500 μg/kg, n = 8) or vehicle (n = 8) via the tail vein on the day of, and 3 and 6 d after the development of ascites. Hemodynamic studies were performed on the day after the final injection.

Isolated aortic ring studies

On the day of the experiment, the thoracic aorta was removed from control rats and cirrhotic animals with ascites and cut into 3-mm rings. The rings were suspended between two triangular stainless steel stirrups in a 20-mL jacketed organ chamber containing modified Krebs-Henseleit solution (118 mmol/L NaCl, 4.6 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 11.1 mmol/L glucose, 27.2 mmol/L $NaHCO_3$, 0.03 mmol/L Na_2 ethyle nediaminetetraacetic acid, 1.8 mmol/L $CaCl_2$) at 37 ℃ and bubbled with 950 mL/L O_2 and 50 mL/L CO_2. The lower stirrup was anchored and the upper stirrup was attached to a force-displacement transducer (TB-652T; Nihon Kohden) to record the isometric force. All rings were

stretched to generate a resting tension of 2 g, which was optimal for contractions of the aortic rings in response to phenylephrine, an α-adrenergic receptor agonist. After 1 h of equilibration, the presence of functional endothelium was determined by the addition of acetylcholine (10 μmol/L). All rings were rinsed and allowed to equilibrate for an additional 1 h in the presence of indomethacin (10 μmol/L) to prevent the influence of the endogenous prostanoids. The aortic rings of the control rats or cirrhotic animals with ascites ($n = 8$, respectively) were incubated for 30 min with anti-AM antibody (1 mg/L) or vehicle. Another control and cirrhotic rings were incubated with either human AM (100 nmol/L) or vehicle in the presence or absence of NG-nitro-L-arginine methyl ester (L-NAME) (30 μmol/L) ($n = 8$, respectively). Then, the cumulative dose-response curves to phenylephrine (1 nmol/L to 10 μmol/L) were evaluated. On completion, the rings were dried and weighed. The force of contraction was expressed as mg of contraction per mg of dried tissue. From each dose-response curve, the maximum response (Rmax) and phenylephrine concentration required for 50% of the maximum response (EC_{50}) were calculated with a nonlinear regression method using computerized curve-fitting software (StatView 5.0 program, Abacus Concept Inc., Berkeley, CA, USA), and were used to compare the contractility and reactivity of phenylephrine-induced contraction, respectively.

Measurements

The AM concentrations in the aorta were measured in the control rats and the cirrhotic animals with/without ascites ($n = 10$, respectively). The aorta was homogenized for 1 min in 10 volumes of 1 mol/L acetic acid and immediately heated at 100 ℃ for 10 min. The homogenates were centrifuged at 15 000 g for 10 minutes at 4 ℃. The supernatants were frozen until analyzed. The AM concentrations were measured by radioimmunoassay[18]. The protein concentrations were determined by Bradford's method[23]. Serum NOx levels were measured as previously described[24].

Chemicals

The human AM and anti-AM antibody were kindly supplied from Diagnostic Science Division, Shionogi & Co., Ltd. (Settsu, Japan). The anti-AM antibody which specifically binds to the C-terminal structure of AM and neutralize the effect of AM, belongs to immunoglobulin G1 subclass and equally cross-reacts with the rat AM [1-50], but not with calcitonin gene-related peptide or amylin[25]. The mouse IgG, KCl, acetylcholine, L-NAME, indomethacin, and phenylephrine were purchased from Sigma Chemical (St. Louis, MO, USA).

Statistic analysis

All analyses were performed with StatView 5.0 program (Abacus Concept Inc.). Comparisons were made using the two-tailed Student's t test for quantitative variables. All data are expressed as mean ± SE. $P < 0.05$ was considered statistically significant.

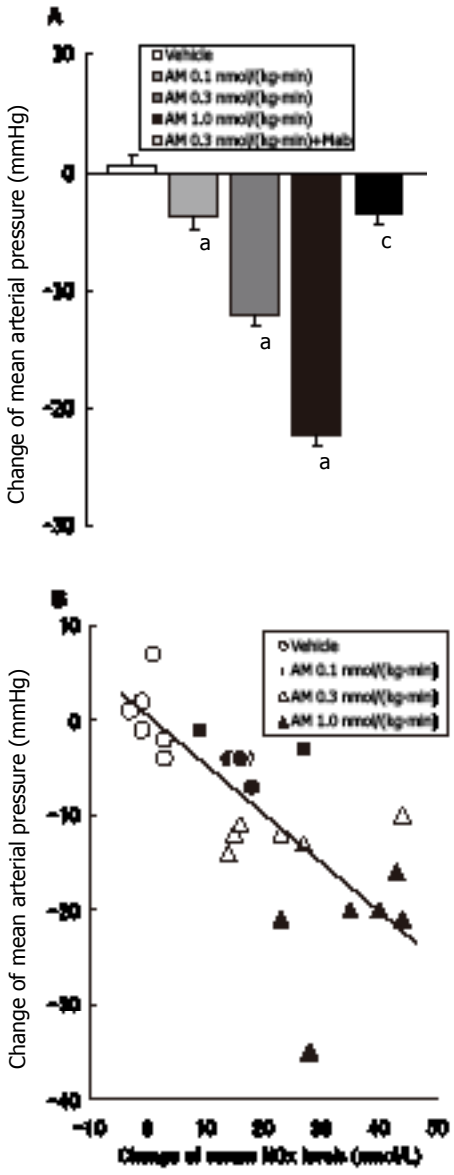

Figure 1 Changes of the mean arterial pressure and serum NOx levels by acute administration of exogenous adrenomedullin. **A**: Arterial pressure; $^{a}P < 0.05$ *vs* vehicle; $^{c}P < 0.05$ *vs* AM 0.3 nmol/(kg·min); **B**: serum NOx levels. mean ± SE. $r = -0.72$, $P < 0.05$, $n = 6$.

RESULTS

Acute administration of exogenous AM

Acute administration of human AM reduced the systemic arterial pressure in the control rats in a dose-dependent manner (Figure 1A, the changes of the mean arterial pressure, vehicle: 0.5 ± 1.6 kPa, AM 0.1 nmol/(kg·min): -3.8 ± 0.8 kPa, $P < 0.05$ *vs* vehicle, AM 0.3 nmol/(kg·min): -12.0 ± 0.6 kPa, $P < 0.05$ *vs* vehicle, AM 1.0 nmol/(kg·min): -22.2 ± 2.7 kPa, $P < 0.05$ *vs* vehicle, respectively). The changes of the mean arterial pressure by AM infusion (0.3 nmol /(kg·min)) were abolished by the pre-treatment with anti-AM antibody (-3.5 ± 0.6 kPa, $P < 0.05$). Moreover, AM infusion increased serum NOx levels in a dose-dependent manner, and the changes of serum NOx levels

Table 1 Hemodynamic effects of chronic administration of adrenomdullin in control rats

	Vehicle ($n=8$)	AM ($n=8$)	P value
Mean arterial pressure (mmHg)	132±3	122±4	<0.05
Cardiac index (mL/min·100g bw)	26.0±2.1	34.1±2.9	<0.05
Systemic vascular resistance (mmHg·min·100g bw/mL)	5.3±0.4	3.8±0.4	<0.05
Portal pressure (mmHg)	6.9±0.5	7.0±0.5	NS
Portal venous inflow (mL/min·100g bw)	3.5±0.4	5.0±0.6	<0.05
Portal venous system resistance (mmHg·min·100g bw/mL)	2.1±0.3	1.6±0.3	NS
Splanchnic arterial resistance (mmHg·min·100g bw/mL)	38.5±4.1	25.7±4.0	<0.05

Control rats were chronically infused with either vehicle or human adrenomedullin (AM) (1.0 mg/h) for 14 d using mini-osmotic pump. On day 14 of chronic infusion, hemodynamic study was performed using radioactive microspheres. Values are presented as mean±SE of 8 separate experiments.

Table 2 Effects of anti-adrenomedullin antibody on hemodynamics and aortic ring contraction of cirrhotic rats.

	Vehicle	Anti-AM antibody
Hemodynamic effects		
Mean arterial pressure (mmHg)	117 ± 3	121 ± 4
Cardiac index (mL/min·100g bw)	39.5 ± 2.9	38.2 ± 3.8
Systemic vascular resistance (mmHg·min·100g bw/mL)	3.1±0.4	3.4±0.4
Portal pressure (mmHg)	13.0 ± 0.4	12.8 ± 0.5
Portal venous inflow (mL/min·100g bw)	6.8 ± 0.8	5.6 ± 1.0
Portal venous system resistance (mmHg·min·100g bw/mL)	2.2 ± 0.3	2.6 ± 0.4
Aortic ring contraction		
Rmax (mg/mg tissue)	998 ± 96	1499 ± 137a
EC50 (nmol/L)	85.7 ± 11.8	53.8 ± 6.2

Cirrhotic rats with ascites were repeatedly injected either vehicle or anti-adrenomedullin (anti-AM) antibody [500 mg/(kg·times)] via tail vein on the day of occurrence of ascites, 3 and 6 d after. Hemodynamic study was performed on the next day of the final injection. Values are presented as mean±SE of 8 separate experiments. The contraction of aortic rings to phenylephrine was evaluated in the presence of vehicle or anti-AM antibody. Values are presented as mean±SE of 8 rings. Rmax: maximal contraction to phenylephrine, EC_{50}: phenylephrine concentration required for 50% of Rmax, $^aP<0.05$ *vs* vehicle.

by AM infusion negatively correlated with the changes of the mean arterial pressure (Figure 1B, $r=-0.72$, $P<0.05$). Exogenous AM reduced the systemic arterial pressure in the cirrhotic rats, as well (vehicle: -0.5±1.8 kPa, AM 0.1 nmol/(kg·min): 0.3±1.9 kPa, AM 0.3 nmol/(kg·min): -4.0±0.6 kPa, AM 1.0 nmol/(kg·min): -13.2±1.7 kPa, $P<0.05$ *vs* vehicle, respectively), but the magnitude of depressor response in the systemic arterial pressure was lower in the cirrhotic rats than in the controls.

Chronic administration of exogenous AM

In agreement with the results of acute administration, chronic administration of exogenous AM caused systemic hypotension as compared with vehicle infusion (Table 1). Chronic infusion of AM increased the cardiac index and reduced the systemic vascular resistance as compared with vehicle infusion. Moreover, chronic AM infusion increased the portal venous inflow and reduced the splanchnic arterial resistance as compared with vehicle infusion. The portal pressure and portal venous system resistance were unchanged by chronic AM infusion.

Effects of anti-AM antibody on hemodynamics and vascular tonus in cirrhotic rats

To evaluate whether the circulating endogenous AM is associated with the circulatory disturbance in cirrhosis, the effects of anti-AM antibody on the hemodynamics were investigated in cirrhotic rats with ascites (Table 2A). Despite the repeated administration of anti-AM antibody that neutralizes the circulating AM, the systemic and splanchnic circulations of the cirrhotic rats were both unchanged. To evaluate whether the endogenous AM in the vascular tissue plays a role in the vascular tonus in the cirrhotic rats, the effects of anti-AM antibody on the phenylephrine-induced contractile response of the control and cirrhotic aortas were evaluated. In the cirrhotic aorta, the anti-AM antibody enhanced the contractility of the phenylephrine-induced contraction without affecting the reactivity as compared with vehicle-treatment (Table 2B). On the other hand, this antibody did affect the contractile response of the control aortas as compared with vehicle.

AM concentrations in the aorta

The AM concentrations in the aorta were higher in the cirrhotic rats than in the controls (Figure 2A, 21.9±2.3 *vs* 12.9±1.2 fmol/mg, $P<0.05$). The cirrhotic rats with

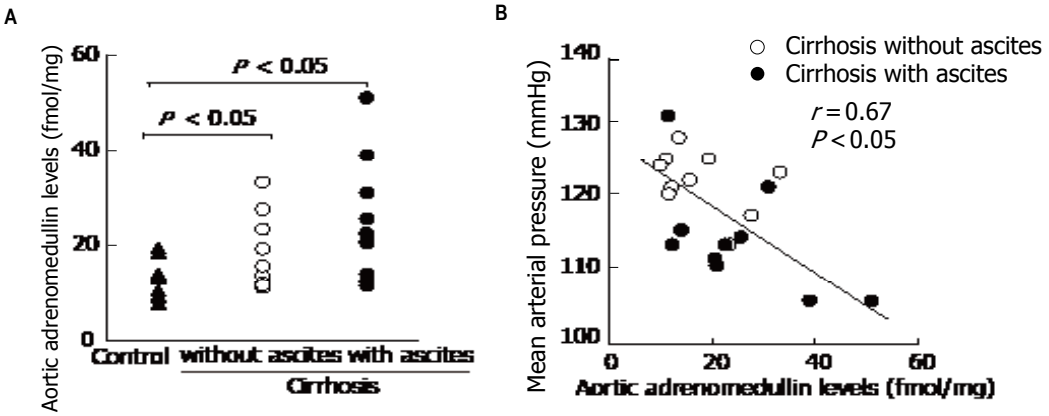

Figure 2 Adrenomedullin concentrations in the aorta in the control and cirrhotic rats (**A**) and their relation with the mean arterial pressure (**B**). ▲ : Control rats, ○ : Cirrhotic rats without ascites, ●: Cirrhotic rats with ascites. mean ± SE. $n = 10$.

Table 3 Contractile response of control and cirrhotic aortic rings to adrenomedullin and/or N^G-nitro-L-arginine methyl ester

	Control		Liver cirrhosis	
	Rmax (mg/mg tissue)	EC_{50} (nmol/L)	Rmax (mg/mg tissue)	EC_{50} (nmol/L)
Vehicle	1860±152	68.8±9.4	998±96[a]	85.7±11.8
AM	1330±118[a]	82.7±12.3	698±94[c]	91.0±12.8
L-NAME	2362±182[a]	61.8±7.1	2274±148[c]	72.1±6.7
L-NAME+AM	2242±91[a]	55.7±9.5	2092±219[c]	53.8±6.2

The contraction of aortic rings to phenylephrine was evaluated in the presence of vehicle or anti-AM antibody. Values are presented as mean±SE of 8 rings. Rmax: maximal contraction to phenylephrine, EC^{50}: phenylephrine concentration required for 50% of Rmax, [a]$P < 0.05$ *vs* vehicle-treated control aorta, [c]$P < 0.05$ *vs* vehicle-treated cirrhotic aorta. AM, adrenomedullin; L-NAME, N^G-nitro-L-arginine methyl ester.

ascites showed the highest aorta AM concentration (24.8 ± 4.0 fmol/mg) which was approximately two-fold increased as compared with that in the controls. Moreover, the AM concentrations in the aorta negatively correlated with the mean arterial pressure in the cirrhotic rats ($r = -0.67$, $P < 0.05$, Figure 2B).

Interaction of AM with NO in phenylephrine-induced contraction of the aortic rings

To investigate the interaction between AM and NO on the vascular tonus, the effect of AM on phenylephrine-induced contraction of the aortic rings was examined in the presence or absence of L-NAME (Table 3). AM-treatment reduced the contractility of the aorta as compared with vehicle-treatment in both of the control and cirrhotic aortas (Rmax: control; 1330 ± 118 *vs* 1860 ± 152 mg/mg tissue, $P < 0.05$, cirrhosis; 698 ± 94 *vs* 998 ± 96 mg/mg tissue, $P < 0.05$), whereas the reactivity showed no difference between AM-treatment and vehicle-treatment (EC_{50}: control; 82.7 ± 12.3 *vs* 68.8 ± 9.4 nmol/L, cirrhosis; 91.0 ± 12.8 *vs* 85.7 ± 11.8 nmol/L). L-NAME potentiated the contractility of both the control and cirrhotic aorta as compared with vehicle without affecting the reactivity (Rmax: control; 2362 ± 182 mg/mg tissue, $P < 0.05$, cirrhosis; 2274 ± 148 mg/mg tissue, $P < 0.05$, EC_{50}: control; 61.8 ± 7.1 nmol/L, cirrhosis; 72.1 ± 6.7 nmol/L). When the aortic rings were pre-treated with L-NAME, AM had no effects on the contractile response of both the control and cirrhotic rings (Rmax: control; 2242 ± 91 mg/mg tissue, cirrhosis; 2092 ± 219 mg/mg tissue, EC_{50}: control; 55.7 ± 9.5 nmol/L, cirrhosis; 53.8 ± 6.2 nmol/L).

DISCUSSION

Arterial vasodilation leading to a low systemic vascular resistance is the most outstanding hemodynamic alteration in the human and murine liver cirrhosis[1-3]. This vasodilation has been suggested to be a major pathogenic mechanism for hyperdynamic circulation characterized by arterial hypotension, hypervolemia, and high cardiac output[1-3]. Although the precise mechanism of the vasodilation in liver cirrhosis remains unknown, the overproduction or reduced degradation of endogenous vasodilators may play a major role. AM, a potent vasodilator peptide, is overproduced by various stimuli including endotoxin, cytokines, vasoactive substances and/or shear stress[26,27], which are enhanced in liver cirrhosis[28,29]. Moreover, the circulating AM levels and the gene expression of AM in the vascular tissues are both elevated in the human and/or murine liver cirrhosis[14-18]. These findings indicate that AM may be implicated in the circulatory disturbance in liver cirrhosis. The aim of this study was to investigate whether the increased circulating AM and/or the enhanced vascular production of AM plays a role in the circulatory disturbance in liver cirrhosis.

Acute administration of exogenous AM to control rats reduced the systemic arterial pressure along with an increase of serum NOx levels in a dose dependent manner, and

the changes of serum NOx levels correlated with the changes of the mean arterial pressure. Moreover, chronic infusion of AM (1.0 μg/h for 14 d) which keeps plasma human AM concentrations within the physiological limit[22] reduced the vascular resistance and increased the blood flow in both of the systemic and splanchnic circulations. These results, together with the existence of AM receptors in the vascular endothelial cells[11], suggest that AM may cause arterial vasodilation via NO synthesis in the vascular wall and lead to a hemodynamic alteration resembling liver cirrhosis.

Whether the circulating endogenous AM is associated with the circulatory disturbance in liver cirrhosis remains to be established. In this study, we used the anti-AM antibody to abolish the effect of the circulating endogenous AM. This antibody possesses an extremely potent neutralizing potency against AM[25], which may allow us to elucidate the role of the endogenous AM in the circulatory disturbance in cirrhosis. Five hundred μg/kg of the anti-AM antibody abolished hypotension following AM infusion at the dose of 0.3 nmol/kg/min for 10 min. Our previous study showed that plasma concentrations of AM in the cirrhotic rats with ascites were 19.2 ± 5.4 fmol/mL[18]. Therefore, the dose of anti-AM used in this study corresponds to that to neutralize the 200-fold of the circulating AM levels of the cirrhotic rats, indicating the enough dose to neutralize the effect of the circulating AM. However, this neutralizing antibody did not affect any hemodynamic parameters in the cirrhotic rats. Moreover, most of the circulating AM is reportedly occupied by glycine-extended AM, an inactive intermediate form of AM, and reflects the process of AM production in the tissue[30]. Therefore circulating endogenous AM may not play a significant role in the circulatory disturbance of liver cirrhosis.

There are no significant difference among the AM levels in plasma samples obtained from the hepatic vein, renal vein, pulmonary artery and femoral artery in cirrhotic patients[15]. The lack of significant arterio-venous difference in the AM levels in various vascular territories suggests that the increased plasma AM levels in cirrhotic patients do not result from an increased production in a specific organ. In this study, the AM concentrations in the aorta were more enhanced in cirrhotic rats than in the controls, and correlated with the mean arterial pressure in the cirrhotic rats. It is, therefore, possible that the endogenous AM contributes to the circulatory disturbance in cirrhosis as a paracrine and/or autocrine regulator of the vascular tonus rather than a circulating hormone. To investigate the role of the endogenous AM in the vascular tonus of cirrhotic rats, we performed isolated aortic ring studies. The cirrhotic aorta showed a blunted contractile response to phenylephrine as compared with the controls. The anti-AM antibody ameliorated the blunted contractile response in the cirrhotic aorta, but did not affect the contraction of the control aorta. This finding, together with the increased AM concentrations in the cirrhotic aorta, indicates that the endogenous AM in the aorta may play a role in the vascular tonus in liver cirrhosis.

It is widely recognized that NO plays a major role in the blunted vascular tonus in cirrhosis[31,32]. Moreover, AM and NO stimulate the synthesis and secretion of each other[11,33]. We, therefore, investigated the interaction between AM and NO in the contractile response of the cirrhotic aorta. In both of the control and cirrhotic aortas, AM reduced the contractile response, but not in the presence of NO synthase inhibitor. Prostanoids are unlikely to explain the blunted contractile response caused by AM, because sufficient indomethacin was used to inhibit cyclooxgenase. These findings, together with an increase in serum NOx levels by the exogenous AM infusion, indicate that AM overproduced in the vascular wall of cirrhotic rats may regulate the vascular tonus via NO synthesis in a paracrine and/or autocrine manner. In liver cirrhosis, an increase in hepatic vascular resistance is the initial phenomenon leading to portal hypertension. This is primarily due to the structural distortion of the hepatic microcirculation caused by cirrhosis, but is also associated with a deficient NO production in the liver, which results in an increased hepatic vascular resistance in contrast to an increased portal inflow via a vasodilation in pre-hepatic (splanchnic) vessels[34]. Considering the interaction between AM and NO, AM may be associated with portal hypertension through an imbalance of the NO production in the hepatic and pre-hepatic vessels.

Interestingly, the magnitude of hemodynamic alteration caused by AM infusion to the control rats was less than that in the cirrhotic animals with ascites, despite the similar hypotension in both groups. It has been considered that the circulatory disturbance in cirrhosis is initially caused by arterial vasodilation and thereafter promoted by an increase in the blood volume resulting from the impaired water and sodium excretion by activation of the endogenous vasoconstrictive systems[1-3]. Because the chronic infusion of AM suppresses the renin activity and aldosterone concentration[22,35], the lack of activation of the vasoconstrictor system may result in a less hemodynamic alteration in the AM-infused rats as compared with the cirrhotic animals. This finding provides further support for the concept that activation of the vasoconstrictive system as well as arterial vasodilation is essential for the circulatory disturbance in liver cirrhosis.

In conclusion, the AM concentrations in the aorta were elevated and negatively correlated with the systemic arterial pressure in the cirrhotic rats. Anti-AM antibody ameliorated the blunted contractile response of the cirrhotic aorta, whereas neutralization of circulating AM by anti-AM antibody did not affect the hemodynamic parameters in cirrhosis. These findings indicate that increased AM production in vascular tissue may contribute to the circulatory disturbance in cirrhosis, acting as a local regulator of the vascular tonus rather than a circulating hormone.

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