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Prevention of hepatopulmonary syndrome and hyperdynamic state by pentoxifylline in cirrhotic rats

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ABSTRACT: Inhibition of tumour necrosis factor-α (TNF-α), levels of which are increased in the blood of cirrhotic rats, prevents hyperdynamic circulatory state, mainly by decreasing the vascular overproduction of nitric oxide. Hepatopulmonary syndrome, which is characterised by intrapulmonary vascular dilatation and increased alveolar to arterial oxygen tension difference ($P_{A\text{-}a,O_2}$), is mainly related to pulmonary overproduction of NO by macrophages accumulated in lung vessels. Since TNF-α is a potent activator of macrophagic inducible nitric oxide synthase (NOS), the aim of this study was to investigate whether TNF-α inhibition prevented hepatopulmonary syndrome and hyperdynamic circulatory state in rats with cirrhosis.

TNF-α was inhibited by 5 weeks of pentoxifylline (10 mg·kg body weight^{-1}·day^{-1}) in rats with cirrhosis induced by common bile duct ligation.

Cardiac output, pulmonary and systemic vascular resistance, $P_{A\text{-}a,O_2}$ and cerebral uptake of intravenous technetium-99m-labelled albumin macroaggregates (which reflects intrapulmonary vascular dilatation) were similar in sham- and pentoxifylline-treated cirrhotic rats. Blood TNF-α concentrations and pulmonary intravascular macrophage sequestration, as assessed by morphometric analysis and radioactive colloid uptake, were decreased with pentoxifylline. Pentoxifylline also prevented increases in aorta and lung NOS activities and inducible NOS expression.

Thus pentoxifylline prevents development of hyperdynamic circulatory state and hepatopulmonary syndrome, probably by inhibiting the effects of tumour necrosis factor-α on vascular nitric oxide synthase and intravascular macrophages. These results support an important role for tumour necrosis factor-α in the genesis of hepatopulmonary syndrome.

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Rats with cirrhosis induced by common bile duct ligation develop both hyperdynamic circulatory state with systemic and splanchnic vasodilatation [1], and hepatopulmonary syndrome [2] with intrapulmonary vascular dilatation and an increased alveolar to arterial oxygen tension difference ($P_{A\text{-}a,O_2}$). These syndromes replicate the systemic and pulmonary vascular abnormalities seen in humans with advanced liver cirrhosis [3]. Studies using nitric oxide synthase (NOS) inhibitors have demonstrated that nitric oxide overproduction in the pulmonary and systemic vasculatures plays a central role in both hyperdynamic circulatory state [4] and hepatopulmonary syndrome [2].

Several observations point to the involvement of tumour necrosis factor-α (TNF-α) in the development of hyperdynamic circulatory state [5–8]. TNF-α is a pro-inflammatory cytokine released by mononuclear cells in response to inflammatory stimuli such as microorganisms or bacterial wall products [9]. Blood TNF-α concentrations are increased in humans and rats with liver cirrhosis [9], and TNF-α inhibition prevents hyperdynamic circulatory state mainly by decreasing vascular NO overproduction [8]. Whether TNF-α is also implicated in the genesis of hepatopulmonary syndrome is unknown.

The overproduction of NO seen in the lungs of cirrhotic rats is ascribable mainly to intravascular macrophage induction of inducible NOS (iNOS) [2], which accumulates in the pulmonary vasculature [10, 11]. Since TNF-α is a potent iNOS activator in macrophages [12], it was hypothesised that TNF-α inhibition might prevent pulmonary NO overproduction, thereby preventing hepatopulmonary syndrome. Pentoxifylline (PTX), a nonspecific phosphodiesterase inhibitor, blocks TNF-α synthesis [13, 14] and TNF-α-induced macrophagic NO production [14–18], by increasing intracellular concentrations of cyclic adenosine 3′,5′-monophosphate and cyclic guanosine 3′,5′-monophosphate. Other reported effects of PTX include inhibition of monocyte chemoattractant protein-1, interleukin-6 and -8, and macrophage inflammatory protein-1a and -1b, decreased expression of adhesion molecules on endothelial cells, decreased activation of neutrophils, decreased proliferation of lymphocytes and monocytes, and decreased binding and transmigration of leukocytes [13–20]. Although PTX is widely used for treatment of peripheral arterial disease, there is increasing evidence that PTX may also play a therapeutic role in the inhibition of the inflammatory process.

For editorial comments see page 661.

The aim of the present study was to determine whether prophylactic PTX treatment in rats with cirrhosis decreased induction of pulmonary intravascular macrophages and NO overproduction in the lungs and systemic vasculature, thereby preventing development of both hepatopulmonary syndrome and hyperdynamic circulatory state.

Methods

The study protocol was reviewed and approved by the Centre Chirurgical Marie Lannelongue (Université Paris Sud, Paris, France) animal care committee. Male Wistar rats were subjected to ligation of the common bile duct to induce cirrhosis, as previously described [2], or a sham operation. PTX was given orally by gavage at a dose of 10 mg·kg body weight^{-1}·day^{-1} for the first 5 weeks after surgery. Four groups of 30 rats were obtained: PTX-treated and untreated cirrhotic rats; and PTX-treated and untreated sham-operated rats.

Haemodynamic and blood gas measurements

After anaesthesia using intraperitoneal ketamine (100 mg·kg body weight^{-1}) and xylazine (0.75 mg·kg body weight^{-1}), the rats were ventilated with room air through a tracheotomy. Catheters were inserted into the pulmonary and tail arteries. A thermistor was positioned in the aortic arch to measure cardiac output. Pulmonary and systemic vascular resistance were calculated by dividing the mean pulmonary and systemic artery pressures by the cardiac index (cardiac output per 100g body weight). PA-a,O_2 was calculated using the modified alveolar gas equation. Haemodynamic and blood gas values were recorded 30 min after the ventilation was adjusted to obtain an arterial carbon dioxide tension (Pa,CO_2) of 4.7–6.0 kPa (35–45 mmHg). At the end of the haemodynamic study, a laparotomy was performed for bacteriological study, measurement of portal venous pressure, blood sampling from the inferior vena cava for asparate transaminase (AST) and alanine transaminase (ALT) measurement, and removal and weighing of the spleen and liver. Twelve rats were studied in each group.

Detection of intrapulmonary vascular dilatation

Technetium-99m-labelled albumin macroaggregates (200 μCi; mean diameter 20 μm, range 15–50 μm) were injected through a jugular catheter. Lung and brain radioactivity were measured using a gamma detector 30 min after the injection, and the brain/lung radioactivity ratio was calculated. Five rats were studied in each group.

Bacteriological studies

A swab of ascitic fluid from the peritoneal cavity was plated on to chocolate agar plates. Blood (3 mL) was withdrawn from the inferior vena cava and inoculated into aerobic and anaerobic Bactec culture bottles (Becton Dickinson, Paris, France). The mesenteric lymph nodes were dissected and plated on to chocolate agar plates. Any positive mesenteric lymph node cultures were considered indicative of bacterial translocation from the intestinal lumen. Ten rats were studied in each group.

Determination of pulmonary intravascular macrophage sequestration

Distended lungs (four rats in each group) were fixed by infusion of 10% formalin at a pressure of 25 cmH_2O into the trachea. Lung sections (4 μm) were stained with haematoxylin and eosin. It has recently been shown that small pulmonary vessels from cirrhotic rats contain adherent large mononuclear macrophage-like cells strongly immunoreactive for the specific rat macrophage monoclonal antibody ED1 [2, 10]. In order to quantify intravascular macrophage sequestration, 60 vessels per animal were examined and the percentage of vessels with >10 mononuclear macrophage-type cells was determined for each group. Only vessels that were circular in cross-section were included.

Scintigraphic imaging of phagocytic function

Lung and liver phagocytosis were assessed as previously described [10], by *in vivo* scintigraphic imaging of the distribution of ^{99m}Tc-labelled tin colloids (1 mCi) injected into the penile vein. The count rates recorded within regions of interest created for the liver and the lung, taken as an index of radioactive colloid uptake for each organ, were used to calculate the lung/liver phagocytic activity ratio. Five rats were studied in each group.

Determination of plasma tumour necrosis factor-α concentration

Plasma TNF-α concentrations were determined using an enzyme-linked immunosorbent assay kit (Biosource International, Camarillo, CA, USA) specific for rat TNF-α. Ten rats were studied in each group.

Nitric oxide synthase activity measurement

Rats were anaesthetised as described above. The thorax was opened, and intravascular lung blood was flushed out by injecting Krebs solution through the pulmonary artery. Lung and aorta samples were frozen in liquid nitrogen and stored at -80°C. The lungs and aortas were placed in ice-cold buffer containing 50 nM tris(hydroxymethyl)aminomethane (Tris)/HCl (pH 7.4) and 1 mM ethylenediamine tetra-acetic acid (EDTA), minced with scissors, homogenised mechanically (Bioblock Scientific, Illkirch, France) and sonicated (Bioblock Scientific) for 20 s on ice. Homogenates were centrifuged for 5 min at 1,000xg at 4°C. The protein concentration of the supernatants was measured, using bovine serum albumin as standard. For total NOS activity determination, 50 mL supernatant (50–100 μg protein) were incubated in a total volume of 160 μL Tris/HCl containing 0.1 mM EDTA, 3 μM tetrahydrobiopterin, 1 mM reduced nicotinamide adenine dinucleotide phosphate, 2.5 mM $CaCl_2$, 5 μM valine and 1 μCi [^{14}C]L-arginine. Duplicate samples were incubated with 5 mM ethyleneglycol tetra-acetic acid (EGTA) for calcium-independent NOS activity determination, and with 0.1 mM L-*N*-nitroso-*N*-methylalanine for NOS-independent arginine to citrulline conversion determination. The reaction was continued for 1 h at 25°C, and then stopped by adding 1 mL stop buffer (3 mM EGTA in ice-cold PBS (pH 5.5)). [^{14}C]L-citrulline was separated by applying the samples to 6.5-cm chromatography columns (Sigma, Saint Quentin Fallavier, France) containing pre-equilibrated home-made DOWEX AGW-X8, and eluting them with 1 mL 1 mM citrulline; radioactivity was measured using a liquid scintillation counter (LS 3801; Beckman, Irvine, CA, USA). Enzyme activity was expressed as picomoles of citrulline produced per milligram of protein per hour. The relationship between protein concentration and [^{14}C]L-citrulline formation was linear in the range 35–125 μg protein (data not shown). In the presence of

0.1 mM L-*N*-nitroso-*N*-methylalanine, arginine to citrulline conversion was <0.1 pmol·mg protein^{-1}·h^{-1} in all experiments. Six rats were studied in each group.

Nitric oxide synthase expression measurement

Expression of endothelial NOS (eNOS) and iNOS in the lungs and aorta was determined using specific antisera directed against eNOS and iNOS (Transduction Laboratories, Lexington, UK), as previously described [2]. Densitometric results for eNOS and iNOS were expressed as a percentage of the value obtained in sham-operated rats (100%). Six rats were studied in each group.

Statistical analysis

Results were evaluated using analysis of variance followed by Fisher's *post hoc* test. Since results were similar in the PTX-treated and untreated sham-operated groups, these were combined for subsequent analyses. All data are presented as mean±SEM. A p-value of <0.05 was considered significant.

Results

Common bile duct ligation induced jaundice in all rats, and liver micronodularity with ascites in most. Gross and histological findings at autopsy showed cirrhosis in all rats subjected to common bile duct ligation, with no difference between the PTX-treated and untreated groups. Blood AST and ALT concentrations, liver and spleen weights, and portal venous pressure were similar in the PTX-treated and untreated cirrhotic groups (table 1). All values were significantly higher than in the sham-operated groups.

Pulmonary intravascular macrophage sequestration

All lungs from PTX-treated and untreated cirrhotic rats showed accumulation of large mononuclear macrophage-like cells within the lumen of numerous small muscular and nonmuscular pulmonary vessels (fig. 1). The percentages of vessels with >10 adherent macrophages was 55±8 in the untreated cirrhotic group but only 27±7 in the PTX-treated cirrhotic group (p<0.04). Mononuclear macrophage cells were not found in pulmonary vessels from sham-operated animals.

Table 1. – Transaminase activity, liver and spleen weight, and portal venous pressure (PVP) in sham-operated rats, and untreated and pentoxifylline (PTX)-treated cirrhotic rats#

	Sham-operated	Cirrhotic	
		Untreated	PTX-treated
AST IU·L^{-1}	62±4	174±23*	144±7*
ALT IU·L^{-1}	25±2	54±9*	43±3*
Liver weight g	14±0.5	27±1*	25±1*
Spleen weight g	1.3±0.1	3.5±0.4*	2.7±0.2*
PVP mmHg	4.2±1.0	7.0±0.7*	8.5±1.5*

Data are presented as mean±SEM. AST: aspartate transaminase; ALT: alanine transaminase. #: 5 weeks after common bile duct ligation. *: p<0.05 *versus* sham-operated rats.

Phagocytic function

Radioactive colloid uptake was virtually confined to the liver in sham-operated animals but predominated in the lungs in untreated cirrhotic animals (fig. 2). PTX decreased lung phagocytic activity (fig. 2), as indicated by the decrease in the lung/liver radioactivity ratio, from 3.5±1.0 in untreated cirrhotic rats to 1.9±0.7 in PTX-treated cirrhotic rats (p<0.05).

Bacteriological studies

Culture-positive mesenteric lymph nodes, indicating that translocation had occurred, were found in 48% of PTX-treated cirrhotic rats and 45% of untreated cirrhotic rats (nonsignificant difference), with similar proportions of cirrhotic rats with positive blood cultures. No bacterial translocation occurred in sham-operated animals.

Plasma tumour necrosis factor-α concentrations

In PTX-treated cirrhotic rats, plasma TNF-α concentrations were intermediate (43±7 pg·mL^{-1}) between those in untreated cirrhotic animals (82±15 pg·mL^{-1}; p<0.05) and those in sham-operated animals (18±8 pg·mL^{-1}; p<0.05).

Hyperdynamic circulatory state

The untreated cirrhotic rats exhibited a characteristic haemodynamic pattern of hyperdynamic circulatory state with pulmonary and systemic arterial vasodilation, as indicated by lower pulmonary and systemic vascular resistances and

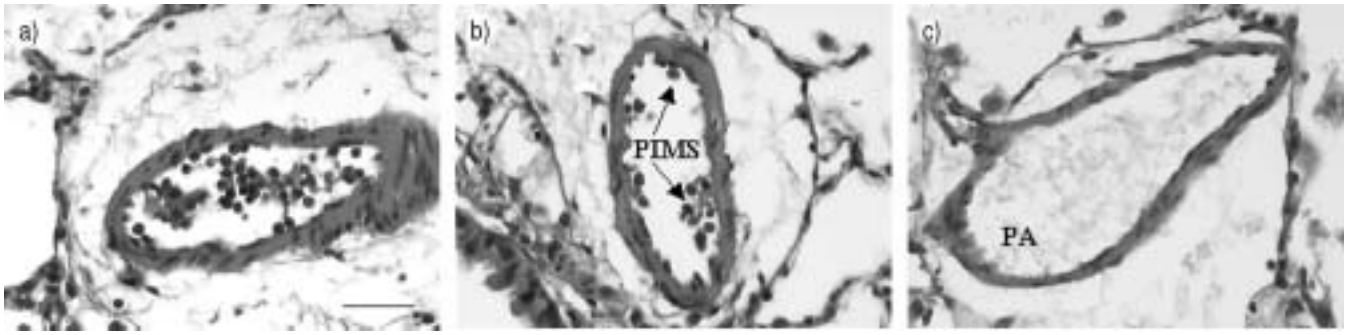

Fig. 1. – Micrographs showing pulmonary intravascular macrophages (PIMS) in the lung of a) an untreated cirrhotic rat and b) a pentoxifylline (PTX)-treated cirrhotic rat. c) No PIMS were found in the lung of a sham-operated rat. The PTX-treated cirrhotic rat showed reduced induction of PIMS. Tissue samples were counterstained with haematoxylin. PA: pulmonary artery. Scale bar=50 μm.

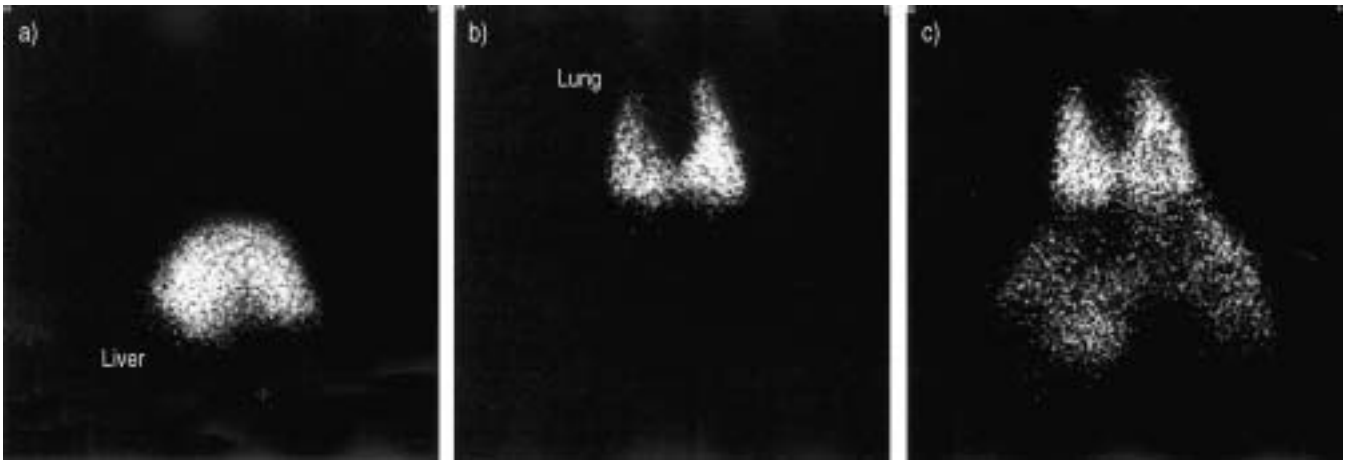

Fig. 2. – Scintigraphic images of the distribution of technetium-99m-labelled tin colloids. Radioactive colloid uptake, an index of phagocytosis, was confined to the liver in sham-operated rats (a) and predominated in the lungs in untreated cirrhotic rats (b). Pentoxifylline decreased lung phagocytic activity in cirrhotic animals (c).

higher cardiac indices compared to the PTX-treated cirrhotic and sham-operated animals (table 2). Conversely, in the PTX-treated cirrhotic rats, pulmonary and systemic vascular resistances and cardiac index were similar to those in the sham-operated animals (table 2), indicating that PTX prevented the development of hyperdynamic circulatory state.

Hepatopulmonary syndrome

The assessment of hepatopulmonary syndrome in the cirrhotic rats was based on a combination of gas exchange abnormalities and intrapulmonary vascular dilatation. P_{a,CO_2} and arterial blood pHs were similar in all groups. In the untreated cirrhotic rats, $P_{A\text{-}a,O_2}$ and brain/lung radioactivity ratio were higher than in the sham-operated and PTX-treated cirrhotic animals, reflecting the presence of intrapulmonary vascular dilatation (fig. 3, table 2). The sham-operated rats and PTX-treated cirrhotic rats exhibited similar $P_{A\text{-}a,O_2}$ and brain/lung radioactivity ratios, indicating that PTX prevented the development of hepatopulmonary syndrome (fig. 3, table 2).

Aortic nitric oxide synthase activity and expression

Total NOS and iNOS activities were higher in the aorta of untreated cirrhotic than in those of sham-operated animals ($p<0.005$) (table 3). PTX-treated cirrhotic rats exhibited aortic total NOS and iNOS activities intermediate between those in untreated cirrhotic rats and sham-operated rats.

eNOS expression in the aorta (fig. 4) increased similarly in PTX-treated and untreated cirrhotic rats compared to sham-operated rats (261±51 and 292±62% of sham value; $p<0.01$).

Compared to sham-operated rats (fig. 4), iNOS expression was greater in the aortas of cirrhotic rats (303±20% of sham value; $p<0.001$) and lesser in PTX-treated cirrhotic rats (58±9% of sham value; $p<0.01$).

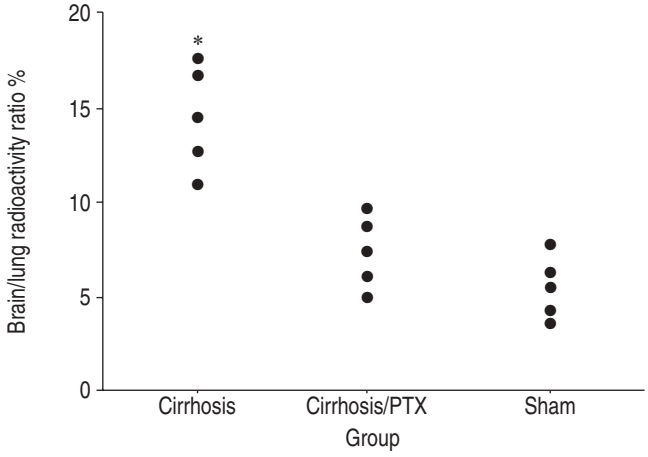

Fig. 3. – Brain/lung radioactivity ratio after intravenous injection of 200 μCi technetium-99m-labelled albumin macroaggregates in untreated cirrhotic rats, pentoxifylline (PTX)-treated cirrhotic rats and sham-operated rats. *: $p<0.05$ *versus* sham-operated and PTX-treated cirrhotic rats.

Table 2. – Haemodynamic parameters, alveolar to arterial oxygen tension difference ($P_{A\text{-}a,O_2}$) and brain/lung radioactivity ratio in sham-operated rats, and untreated and pentoxifylline (PTX)-treated cirrhotic rats[¶]

	Sham-operated	Cirrhotic	
		Untreated	PTX-treated
Cardiac index $mL \cdot min^{-1} \cdot 100\ g\ body\ weight^{-1}$	36±2#	75±9*	39±3#
Systemic vascular resistance $mmHg \cdot mL^{-1} \cdot min^{-1} \cdot 100\ g\ body\ weight^{-1}$	2.3±0.2#	1.2±0.2*	2.4±0.3#
Total pulmonary vascular resistance $mmHg \cdot mL^{-1} \cdot min^{-1} \cdot 100\ g\ body\ weight^{-1}$	0.50±0.04#	0.23±0.05*	0.45±0.06#
Brain/lung radioactivity ratio %	5±0.4#	14±0.9*	7±1.0#
$P_{A\text{-}a,O_2}$ mmHg	14±4#	28±5*	12±4#

Data are presented as mean±SEM. ¶: 5 weeks after common bile duct ligation. *: $p<0.05$ *versus* sham-operated rats; #: $p<0.05$ *versus* untreated cirrhotic rats. 1 mmHg=0.133 kPa.

Table 3. – Total nitric oxide synthase (NOS) and inducible NOS (iNOS) activities in the aortas and lungs of sham-operated rats, and untreated and pentoxifylline (PTX)-treated cirrhotic rats[¶]

	Sham-operated	Cirrhotic	
		Untreated	PTX-treated
Aorta			
Total NOS pmol·mg protein^{-1}·h^{-1}	2.15±0.25[¶]	6.50±0.65*	4.31±0.46*,[¶]
iNOS pmol·mg protein^{-1}·h^{-1}	0.70±0.17[¶]	1.83±0.27*	1.46±0.32*
Lung			
Total NOS pmol·mg protein^{-1}·h^{-1}	4.5±0.6[¶]	12.4±1.6*	7.65±0.8*,[¶]
iNOS pmol·mg protein^{-1}·h^{-1}	2.2±0.4[¶]	8.6±1.9*	2.8±0.3[¶]

Data are presented as mean±SEM. [¶]: 5 weeks after common bile duct ligation. *: p<0.05 *versus* sham-operated rats; [¶]: p<0.05 *versus* untreated cirrhotic rats.

Lung nitric oxide synthase activity and expression

Total NOS and iNOS activities were higher in the lungs of untreated cirrhotic than in those of sham-operated animals (p<0.05) (table 2, fig. 5). PTX-treated cirrhotic rats exhibited lung total NOS and iNOS activities intermediate between those in untreated cirrhotic rats and sham-operated rats.

eNOS expression in the lungs (fig. 5) showed a larger increase (p<0.05) in PTX-treated than in untreated cirrhotic rats (620±60 and 420±45% of sham value; p<0.01 for both comparisons).

Compared to sham-operated rats (fig. 5) iNOS expression was greater in the lungs of cirrhotic rats (400±44% of sham value, p<0.001) and similar in PTX-treated cirrhotic rats (80±32% of sham value).

Discussion

The present study shows that chronic administration of the TNF-α inhibitor PTX prevents development of both hepatopulmonary syndrome and hyperdynamic circulatory state and reduces lung and aortic NO production in cirrhotic rats. These findings support the hypothesis that TNF-α plays a central role in the genesis of hepatopulmonary syndrome and hyperdynamic circulatory state and that this effect is mediated chiefly by overproduction of NO.

As previously reported in humans [9, 21, 22] and animals [5, 6] with liver cirrhosis, blood TNF-α concentrations were elevated in the present cirrhotic rats. TNF-α is a pro-inflammatory cytokine released by macrophages in response to inflammatory stimuli such as microorganisms or their endotoxins [5, 9, 21]. Recent studies indicate that bacterial translocation may play a significant role in this enhanced TNF-α production in cirrhotic rats [5], by releasing endotoxin, as suggested by the significant correlation found between plasma levels of endotoxin and TNF-α [6]. In the present study, the decrease in blood TNF-α concentration in the rats treated with PTX was not related to a lower incidence of bacterial translocation, which was similar in the treated and untreated cirrhotic rats. A more likely mechanism is the inhibition of macrophage production of TNF-α by PTX, as previously demonstrated *in vitro* and *in vivo* [13–18].

Recent studies support a specific role for TNF-α in promoting the systemic vasodilatation that complicates cirrhosis and/or portal hypertension in rats [5–8, 23]. TNF-α inhibition by thalidomide [24] or tyrphostin [7] or TNF-α antagonism by specific antibodies directed against TNF-α [8] prevents the development of hyperdynamic circulation in portal vein-ligated or cirrhotic rats. In keeping with these studies, it was found in the present study that PTX-treated cirrhotic rats exhibited cardiac indices and systemic as well as pulmonary vascular resistances similar to those found in the sham-operated animals, indicating that TNF-α inhibition by PTX

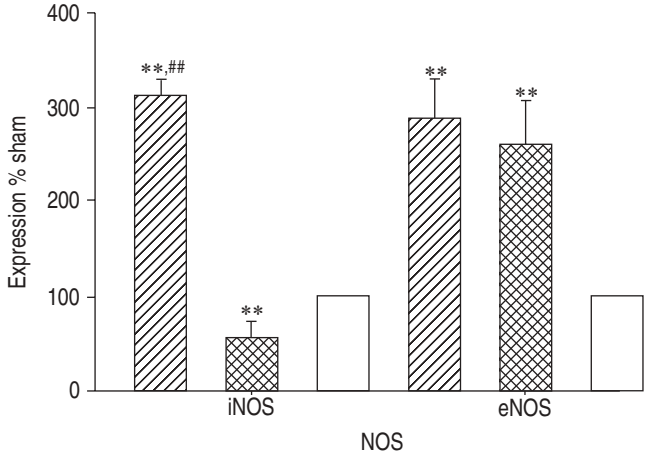

Fig. 4. – Inducible nitric oxide synthase (NOS) (iNOS) and endothelial NOS (eNOS) expression in aorta homogenates from untreated (▨) and pentoxifylline (PTX)-treated (■) cirrhotic rats and sham-operated rats (□). Data were determined densitometrically and are presented as mean±SEM. **: p<0.01 *versus* sham-operated rats; ##: p<0.01 *versus* PTX-treated cirrhotic rats.

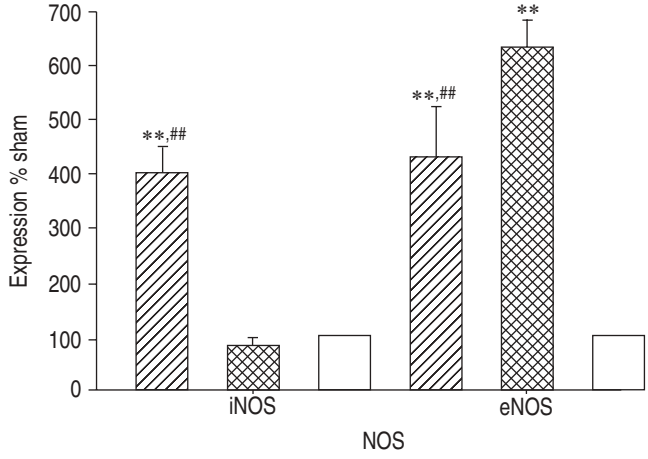

Fig. 5. – Inducible nitric oxide synthase (NOS) (iNOS) and endothelial NOS (eNOS) expression in lung homogenates from untreated (▨) and pentoxifylline (PTX)-treated (■) cirrhotic rats and sham-operated rats (□). Data were determined densitometrically and are presented as mean±SEM. **: p<0.01 *versus* sham-operated rats; ##: p<0.01 *versus* PTX-treated cirrhotic rats.

also prevents development of hyperdynamic circulatory state in cirrhotic rats.

Although several studies have shown that TNF-α inhibition is associated with decreased vascular release of NO [7, 8, 24], a major mediator of TNF-α-induced hyperdynamic circulatory state [8], the effects of TNF-α inhibitors on vascular eNOS and iNOS in cirrhosis need to be clarified. Various groups have demonstrated that iNOS and eNOS are overexpressed in the aorta of cirrhotic rats and that this leads to increased aortic production of NO [25–27]. Overexpression of aortic eNOS is related to increased shear stress in cirrhosis [25], whereas iNOS expression is induced by inflammatory mediators such as TNF-α [12]. However, TNF-α also enhances eNOS activity by stimulating eNOS phosphorylation [28] and upregulating the key eNOS cofactor tetrahydrobiopterin [5]. In agreement with these effects of TNF-α, its inhibition by PTX did not lower aortic eNOS expression but decreased aortic NOS activity and normalised aortic iNOS expressions in the present cirrhotic animals.

In a previous study of cirrhotic rats, it was found that pulmonary NO production was increased mainly in relation to iNOS overexpression in macrophages sequestered in pulmonary microvessels, and that inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) of this pulmonary NO overproduction prevented hepatopulmonary syndrome [2]. It was also shown that translocation of Gram-negative pathogens from the gut to the pulmonary circulation increased this macrophage sequestration [29], probably by inducing coordinated expression of macrophage and endothelium adhesion molecules, as well as local release of cytokines such as TNF-α and monocyte chemotactic factors. In the present study, PTX-treated cirrhotic rats exhibited similar values to those found in sham-operated rats for $P_{A\text{-}a,O_2}$ and the brain/lung radioactivity ratio (the two criteria for hepatopulmonary syndrome). PTX also decreased lung NOS activity, suggesting that PTX prevented hepatopulmonary syndrome by decreasing lung NO production, as in the previous study using L-NAME [2]. This was probably ascribable to both a decrease in the number of pulmonary intravascular macrophages and direct inhibition by PTX of the effects of TNF-α on lung iNOS and eNOS [16]. Decreased macrophage sequestration in the pulmonary vessels was demonstrated directly by a reduction in the number of pulmonary intravascular macrophages determined by quantitative morphometric analysis and indirectly by a decrease in lung radiocolloid uptake, an index of lung phagocytic function (fig. 2). PTX probably inhibits macrophage sequestration by both decreasing the release of TNF-α by the stimulated macrophages [13, 14] and opposing the effects of TNF-α on adhesion molecule upregulation [19] and CC chemokine induction [20].

Compared to sham-operated rats, the levels of iNOS expression and activity in untreated cirrhotic rats increased five- and four-fold respectively in the lung, and only 2.5- and three-fold in the aorta. This difference in NOS induction was further illustrated by the higher ratio of inducible to total NOS activity in the lung (70%) compared to the aorta (30%). The accumulation of pulmonary intravascular macrophages most probably explains the greater inducibility of NOS in the lung, as compared to systemic vessels, where the major source of iNOS is smooth muscle cells [30]. Similar observations have been made for the heart of cirrhotic rats [31].

The effects of PTX on NOS were similar in the lung and aorta and consisted of inhibition of iNOS expression and activity and a reduction in eNOs activity but not expression. Indeed, although pulmonary and systemic haemodynamic parameters returned to normal in the present cirrhotic rats treated with PTX, eNOS expression remained elevated in both the aorta and the lungs. This contrasts with the results of a previous study, in which normalisation by propranolol of systemic haemodynamics in rats with liver cirrhosis was associated with reductions in aortic eNOS expression and activity [25]. The causes of the persistent aortic and lung eNOS overexpression are unclear. It may result from end-product feedback regulation by NO, as observed in the previous study using L-NAME [2]. Additionally, PTX might suppress the downregulatory effect of TNF-α on e-NOS expression [32, 33].

Whether the observed prevention of hepatopulmonary syndrome and hyperdynamic circulatory state was related only to the reduction in NO production remains questionable, since PTX may have decreased the production of other factors that mediate TNF-α effects [8] or directly decreased the inflammatory response. Moreover, factors other than the anti-inflammatory effect of PTX may contribute to the prevention of hyperdynamic circulatory state and hepatopulmonary syndrome. PTX might improve gas exchange by restoring the hypoxic pulmonary vasoconstriction in cirrhotic rats. It might influence the development of portal hypertension and the natural history of obstructive hepatic injury, thus altering the production and/or metabolism of other mediators by the liver. This hypothesis is ruled out, however, by the fact that, as previously reported [34–37], the severity of hepatic injury and portal hypertension were similar in the PTX-treated and untreated cirrhotic rats .

The present study has important implications. It supports prophylactic treatment with PTX in liver cirrhosis, in keeping with prospective studies in which PTX therapy decreased the risk of hepatorenal syndrome and improved survival in patients with severe alcoholic hepatitis [38]. Another potential beneficial effect may be the prevention and/or treatment of hyperdynamic circulatory state and hepatopulmonary syndrome.

The present study shows that prophylactic administration of the tumour necrosis factor-α inhibitor pentoxifylline prevents the development of both hepatopulmonary syndrome and hyperdynamic circulatory state, probably by inhibiting the effects of tumour necrosis factor-α on nitric oxide synthase and intravascular macrophages. These findings confirm the important role of tumour necrosis factor-α in hyperdynamic circulatory state and support its implication in the induction of pulmonary intravascular macrophages and genesis of hepatopulmonary syndrome in cirrhotic rats.

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