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Potential clinical targets in hepatopulmonary syndrome: lessons from experimental models

Sarah Raevens^{1,2} and Michael B. Fallon²

¹Department of Gastroenterology and Hepatology – Hepatology Research Unit, Ghent University – Ghent University Hospital, Ghent, Belgium

²Department of Internal Medicine, University of Arizona College of Medicine, Phoenix, Arizona, USA

Abstract

Hepatopulmonary syndrome (HPS) is a relatively common and potentially severe pulmonary complication of cirrhosis with increased risk of mortality. In experimental models, a complex interaction between pulmonary endothelial cells, monocytes and the respiratory epithelium, producing chemokines, cytokines, and angiogenic growth factors underlies alterations in the alveolar microvasculature, resulting in impaired oxygenation. Models systems are critical for evaluating mechanisms and for preclinical testing in HPS, due to the challenges of evaluating the lung in the setting of advanced liver disease in humans. This review provides an overview of current knowledge and recent findings in the rodent common bile duct ligation model of HPS, which recapitulates many features of human disease. We focus on the concepts of endothelial derangement, monocyte infiltration, angiogenesis, and alveolar type II cell dysfunction as main contributors and potential targets for therapy.

Keywords

intrapulmonary vasodilation; intrapulmonary shunts; cirrhosis; portal hypertension

Pulmonary vascular abnormalities, including diffuse or localized dilations of pulmonary pre-capillary and capillary vessels (intrapulmonary vascular dilations, IPVDs), and intrapulmonary arteriovenous communications, are common in patients with advanced liver disease, with an estimated prevalence of 13% to 57% (1,2). Up to 30% of patients with cirrhosis and IPVDs develop abnormal gas exchange and hypoxemia, which is defined as a hepatopulmonary syndrome (HPS) (3). Three mechanisms are responsible for impaired oxygenation of venous blood, resulting in hypoxemia. First, IPVDs lead to increased perfusion relative to local ventilation (V/Q mismatch), which may be exacerbated by impaired hypoxic pulmonary vasoconstriction (4,5). Second, an increased capillary diameter

Contact information. Sarah Raevens, MD, Department of Gastroenterology and Hepatology, Ghent University Hospital, Building K12 First Floor IE, De Pintelaan 185, 9000 Ghent, Belgium, Phone: +32 9 332 48 81, Fax: +32 9 332 49 84, sarah.raevens@ugent.be, Michael B. Fallon, MD, Department of Internal Medicine, University of Arizona College of Medicine, 550 E. Van Buren Street, Phoenix, Arizona 85004, USA, Phone: + 1 602-827-2078, mfallon@email.arizona.edu.

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lengthens the distance oxygen molecules have to traverse to bind hemoglobin, and may be exacerbated by the hyperdynamic circulation of cirrhosis (diffusion restriction). Third, direct arteriovenous communications allow blood to bypass pulmonary oxygenation and results in admixture of arterial and venous blood. HPS is associated with worse quality of life and significantly increases mortality in affected patients (3). Currently, liver transplantation is the only curative treatment, highlighting the need for novel effective medical therapies.

The development of medical therapies for HPS is dependent on preclinical studies using animal models that share features of human disease. Over the last two decades, a number of rodent models have been tested for the ability to recapitulate the pulmonary vascular and gas exchange abnormalities of human HPS, including common bile duct ligation (CBDL) cirrhosis, thioacetamide (TAA) cirrhosis, and isolated portal hypertension (PH) induced by partial portal vein ligation (PPVL). Of these models, CBDL in the rat is unique in mimicking the physiologic features of human HPS and has been most studied (6). More recently, the characterization of CBDL in the mouse as a model of HPS, albeit with a slight difference in the time course of development of liver injury and HPS relative to the rat, adds significantly to the potential to study pathogenesis (7). During CBDL, the common bile duct is ligated and dissected, resulting in obstructive cholestasis, bile duct proliferation, hepatic inflammation and peri-portal fibrosis. Within one week after CBDL, portal venous pressure begins to rise, followed shortly thereafter by the development of a hyperdynamic circulation (8,9). Intrapulmonary shunting and HPS develop by week 2, when bridging fibrosis is present in the liver, and worsen during the progression to a cirrhotic state (6–8). Cirrhosis develops within 4 weeks in rats and 6 weeks in mice (9). Similar to the human situation, experimental HPS does not require the presence of advanced cirrhosis to develop (3).

Four major pulmonary alterations, intrapulmonary vasodilation, intravascular monocyte infiltration, angiogenesis and alveolar type II (AT2) cell dysfunction have been found to drive gas exchange abnormalities in experimental HPS. This review summarizes the progress that has been made in understanding the mechanisms for these alterations, and in uncovering novel targets for therapy.

Pathogenesis of HPS: lessons from experimental models

Intrapulmonary vascular dilations

Vascular tone—A key feature of HPS is the development of IPVDs. One putative contributor is increased levels of exhaled nitric oxide (NO), a potent vasodilator, which are found in cirrhotic patients with HPS and normalize as the syndrome resolves after liver transplantation (10–12). Similar findings have been found in experimental studies of HPS, where increased exhaled and intrapulmonary NO levels are found after CBDL. In this setting, NO inhibition with N-nitroarginine methyl ester (L-NAME) decreases intrapulmonary shunting and improves gas exchange (13,14). *In vitro* experiments, investigating the contractile response to phenylephrine, confirm that increased pulmonary NO production is responsible for decreased pulmonary vascular tone in experimental HPS (15). The increase in pulmonary NO production results from both increased activity of endothelial nitric oxide synthase (eNOS), produced by the pulmonary microvascular endothelium (16), and inducible nitric oxide synthase (iNOS) produced by infiltrating

monocytes. Downstream, NO diffuses across the vascular smooth muscle cell membrane and activates the guanylate cyclase (GC)/cyclic guanosine monophosphate signaling pathway, leading to vascular relaxation. In this regard, intravenous (IV) methylene blue, which blocks stimulation of GC by NO, has been used anecdotally to treat severe post-transplant hypoxemia in HPS patients (17).

Endothelin-1-induced endothelial dysfunction—Hepatic and plasma endothelin-1 (ET-1) levels increase beginning 1 week after CBDL in experimental HPS, in association with a subsequent (week 2 after CBDL) increase in pulmonary microvascular eNOS, development of IPVDs and the onset of HPS (8,16,18). ET-1 classically acts as a potent vasoconstrictor, including in the portal circulation where it increases sinusoidal and pre-sinusoidal resistance. Paradoxically, circulating ET-1 specifically promotes vasodilation in the pulmonary circulation of CBDL rats. This differential effect of ET-1 depends on the expression of and binding with its receptors. The endothelin A (ET_A) or B (ET_B) receptors expressed by vascular smooth muscle cells mediate vasoconstriction, while the ET_B receptor on endothelial cells upregulates eNOS and NO, mediating vascular relaxation (8). CBDL leads to a specific upregulation of the endothelial ET_B receptor, postulated to be driven by increased pulmonary shear stress derived from increased blood flow during the development of a hyperdynamic circulatory state (19,20). Selective inhibition of the ET_B receptor ameliorates experimental HPS and confirms a central role of the ET-1/ET_B/eNOS/NO pathway in modulating vasodilation (8,21). Interestingly, pulmonary ET_B receptor levels are also increased in PPVL rats, although HPS does not develop in this model, where circulating ET-1 levels are not elevated (8,22). However, exogenous ET-1 can drive the development of HPS in PPVL animals (20). Under normal circumstances, endothelial cells and stellate cells are the major hepatic cell types recognized to produce ET-1 (23). In the CBDL model, the major increase in hepatic ET-1 is derived from proliferating cholangiocytes and results in a selective increase in circulating ET-1 levels relative to other models (PPVL and TAA) (23). Elevated circulation of ET-1 then drives ET_B receptor activation (8,18,23,24). Based on these observations, ET_B receptor blockade may be of potential benefit in HPS.

Monocyte infiltration—A second contributor to NO production after CBDL is iNOS. Multiple studies have found increased pulmonary iNOS levels beginning 3 weeks after CBDL, produced by pulmonary intravascular monocytes which infiltrate the lung vasculature specifically after CBDL (8,13,25–27). Macrophage depletion studies, using clodronate and gadolinium, confirm a reduction in iNOS and improved gas exchange, and support the importance of intravascular monocyte influx in experimental HPS (27). Infiltrating monocytes also express heme oxygenase-1 (HO-1) and produce carbon monoxide (CO) beginning 3 weeks after CBDL, exerting additional vasodilator effects relative to NO (8,26). In human HPS, carboxyhemoglobin levels are increased relative to non-HPS cirrhosis patients, consistent with increased CO production in human disease (28).

Although the importance of pulmonary intravascular monocytes is recognized in experimental HPS (13,26,27,29), little is known about the origin, behavior, and phenotype of these cells. Recruited monocytes are generally considered to be derived from progenitor cells in the bone marrow that migrate to the site of injury. However, a recent study found that

splenic reservoir monocytes contribute to pulmonary monocyte accumulation in experimental HPS (30). Splenectomy decreased pulmonary monocyte influx and improved gas exchange, but increased hepatic monocyte levels and significantly worsened liver function (30).

One trigger for pulmonary intravascular monocyte accumulation appears to be bacterial translocation from the gut into the portal circulation. In the setting of cirrhosis, including CBDL, intestinal bacterial overgrowth, disruption of the gut mucosal barrier, decreased clearance capacity of the liver, and the development of portosystemic shunts allow circulating endotoxins to enter the pulmonary circulation (25,27,31,32). Once there, these bacterial products induce local production of chemotactic factors and cell adhesion molecules, guiding monocyte recruitment and adherence to the pulmonary endothelium (25,31,33). Bacterial translocation, as reflected by culture-positive mesenteric lymph nodes, occurs in the majority of CBDL rats once cirrhosis develops, and correlates with higher incidence and severity of HPS (29,31,32). In contrast to CBDL, where endotoxemia correlates with HPS severity, plasma endotoxin levels are not elevated in PPVL and TAA treated rats where HPS does not develop (24,34,35). Moreover, prophylactic norfloxacin treatment reduces experimental HPS severity and intrapulmonary monocyte recruitment in association with reduced translocation of Gram-negative organisms (29).

Plasma concentrations of tumor necrosis factor alpha (TNF α), a pro-inflammatory cytokine secreted in response to inflammatory stimuli, including endotoxin exposure, are also increased in human and experimental cirrhosis, and parallel circulating endotoxin levels (24,25). Prophylactic TNF α inhibition reduces the development of HPS features in CBDL rats (25,29,36,37). Both pentoxifylline, a non-specific phosphodiesterase inhibitor known to reduce TNF α synthesis, and anti-TNF α antibodies appear to mitigate HPS through prevention or reduction of the development of the hyperdynamic circulation (25,37). Pentoxifylline administration in established experimental HPS also improves gas exchange by down-regulating pulmonary ET $_B$ receptor expression and subsequent eNOS-mediated NO production, without altering systemic or portal pressures (36). *In vitro* experiments support that pentoxifylline inhibits ET $_B$ receptor expression by blocking shear stress mediated upregulation (36).

Chemotactic factors, upregulated by inflammatory cytokines, regulate the recruitment and sequestration of monocytes to the pulmonary vascular bed. Increased pulmonary microvascular expression of fractalkine (CX3CL1) develops after 2 weeks of CBDL and attracts circulating monocytes that display the fractalkine receptor (CX3CR1) and increased CXCR2, a common receptor for various chemokines (38,39). Early pulmonary vascular monocyte recruitment is also found in HPS mice driven by elevated levels of monocyte chemoattractant protein 1 (MCP-1), a main mediator for monocyte attraction, produced by both endothelial cells and macrophages (7,40). Blocking monocyte chemotaxis has been shown to be beneficial, with both CX3CR1 neutralizing antibodies and CXCR2 inhibition reducing intrapulmonary monocyte accumulation and improving experimental HPS (38,39). Downstream, CX3CL1 and CXCR2 signaling have been implicated in direct activation of the Raf/MEK/ERK and PI3K/Akt/eNOS pathway in endothelial cells, promoting cell survival, proliferation and angiogenesis (38,39). Furthermore, ET $_B$ receptor activation by

ET-1 on the pulmonary microvascular endothelial cell augments CX3CL1 production, supporting a mechanistic link between the ET-1/ET_B receptor axis and CX3CL1/CX3CR1 in mediating monocyte adhesion and angiogenesis in experimental HPS (41).

Intrapulmonary arteriovenous shunt formation

Several lines of evidence in experimental and human HPS support that vascular remodeling, endothelial dysfunction and angiogenesis (new blood vessel formation from pre-existing vessels) contribute to HPS development. In humans, single nucleotide polymorphisms in genes involved in the regulation of angiogenesis, including Endoglin (ENG) and von Willebrand Factor (vWF), are associated with the risk of HPS, and circulating levels of these factors, as well as vascular cellular adhesion molecule 1 (VCAM-1), are increased in serum of cirrhotic patients with HPS compared to those without HPS (7,42,43). In CBDL animals, scanning electron microscopic evaluation of pulmonary vascular casts showed, in addition to crypts representing sites of intravascular macrophage adhesion, increased microvascular density and capillary diameter, along with signs of intussusceptive angiogenesis and the presence of direct vascular communications (44). Later studies confirmed increased pulmonary expression of the endothelial markers vWF, VE-cadherin and ENG, supporting the importance of angiogenesis in microvascular proliferation and shunt formation in experimental HPS (7,45).

Angiogenesis is mediated by angiogenic growth factors. Vascular endothelial growth factor A (VEGF-A, present at week 2), platelet-derived growth factor (PDGF, present at week 4), and placental growth factor (PIGF, present at week 2) are all angiogenic factors produced in part by intravascular monocytes, and implicated in pulmonary angiogenesis in experimental HPS. These factors may serve as potential targets for therapy (7,27,45). VEGF-A, PDGF and PIGF stimulate cellular processes by binding to tyrosine kinase receptors on the cell surface. VEGF-A and PIGF belong to the VEGF family. VEGF-A binds to VEGF receptor 1 (VEGFR1) and 2 (VEGFR2), with VEGFR2 being responsible for pro-angiogenic signaling, while VEGFR1 functions as a decoy (46). PIGF specifically binds to VEGFR1 and stimulates angiogenesis both indirectly by displacement of VEGF-A from VEGFR1, triggering VEGF/VEGFR2 signaling, and directly by VEGFR1 activation and stimulation of crosstalk between VEGFR1 and 2 (46). Administration of pentoxifylline, which reduces pulmonary monocyte infiltration, or delivery of recombinant adeno-associated virus-6 encoding human endostatin plus angiostatin which directly blocks angiogenic pathways, downregulated pulmonary angiogenesis and improved HPS in CBDL rats (45). Treatment with sorafenib, a multi-specific tyrosine kinase receptor inhibitor targeting the VEGF and PDGF receptor and Raf family kinases, also reduced the degree of shunting and improved gas exchange in HPS rats (47). The beneficial effect of sorafenib may be attributed to at least three mechanisms. First, sorafenib might indirectly mitigate HPS by improving underlying liver injury, fibrosis and PH (48). Second, sorafenib directly targets the VEGF/VEGFR-2/Akt pathway and pulmonary angiogenesis in the pulmonary microvasculature *in vivo* and in rat pulmonary microvascular endothelial cells *in vitro* (47,48). Third, sorafenib blocks cholangiocyte proliferation and ET-1 production, resulting in decreased eNOS-mediated vasodilation (48). Based on these preclinical data, a pilot randomized clinical trial (RCT) of sorafenib in human HPS has recently completed enrollment (NCT02021929).

The recent identification of PlGF as a central contributor to the development of HPS in mice parallels mechanisms in rat HPS and expands potential therapeutic targets (7). In addition to upregulation of VEGF signaling, PlGF has a direct pro-survival effect on endothelial cells and also attracts VEGFR1-expressing macrophages and bone marrow progenitors, which further stimulate the angiogenic process by additional growth factor release (46). Previous studies revealed that PlGF is not required for physiological vessel maintenance in healthy mice (46,49). However, in the setting of pathology, PlGF can drive angiogenesis (46,49). PlGF neutralization selectively inhibits pathological angiogenesis, and does not cause significant side effects (46,49). Anti-PlGF monoclonal antibodies improve HPS in mice by counteracting pulmonary angiogenesis and by attenuating pulmonary monocyte influx (7). Increased PlGF expression appears to be confined to the lung, predominately in infiltrating monocytes and AT2 cells, and anti-PlGF therapy does not affect liver alterations, supporting its capacity to selectively target the pulmonary compartment (7). Anti-PlGF antibody treatment represents an attractive drug candidate, and has been found to be safe (49) and well-tolerated in human healthy volunteers and oncologic patients (50–52).

Together, findings in rat and mouse HPS support that pulmonary angiogenesis is a key feature and an important potential therapeutic target in both situations and in humans. Moreover, angiogenesis in experimental HPS appears to be a consequence of both a pulmonary pro-inflammatory state, with infiltrating monocytes producing pro-angiogenic mediators (7,27,45), as well as inflammatory and vasoactive mediators directly affecting the pulmonary endothelium (53–56). Therefore, a broad range of targets, including the gut microbiome, bacterial translocation, chemokine signaling, cell adhesion molecule expression, monocyte activation as well as direct inhibition of vasoactive and angiogenic mediators could have beneficial effects in human disease.

Involvement of the respiratory epithelium

Generally, HPS has been considered a disorder of the alveolar microcirculation with definitions excluding both significant intrinsic lung disease and ventilation abnormalities. However, recent findings have challenged this view. For instance, it is well recognized that HPS may co-exist with intrinsic lung abnormalities although the two processes have been considered distinct. More recently, subtle pulmonary function test abnormalities including lower forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) with preserved FEV1/FVC ratio, have been observed in cirrhotic patients with HPS compared to those without, supporting that ventilation defects may contribute to gas exchange abnormalities in HPS (57).

Similar findings are found in experimental HPS, where reductions in tidal volume, minute ventilation and mean inspiratory flow rate, attributable to uneven distribution of alveolar ventilation and decreased mean alveolar cord length reflecting alveolar collapse have been found (58). These functional and structural alveolar alterations are associated with increased apoptosis of AT2 cells and a resultant decrease in surfactant protein (SP) production (SP-A, B, C, D) (57,59). Surfactant, a lipoprotein complex formed by AT2 cells, maintains alveolar integrity and prevents collapse, and also regulates lung injury and inflammation. Elevated circulating bile acid and TNF α levels, found after CBDL, may contribute to effects on AT2

cells as exposure to chenodeoxycholic acid, the bile acid nuclear receptor agonist GW4064 or TNF α at physiologic concentrations induced apoptosis and SP protein loss in cultured AT2 cells (57). Moreover, AT2 cell integrity and SP production are maintained in PPVL animals, in which circulating bile acids and TNF α levels are not elevated. Interestingly, serum bile acids are significantly increased in cirrhotic patients with HPS compared to patients without HPS (60). These observations, together with our knowledge that experimental HPS exclusively develops in a model of cholestatic liver disease, and not following PPVL or TAA, suggests that altered bile acid signaling may contribute to HPS development. The mechanistic link between cholestasis, AT2 cell dysfunction and HPS has not been unraveled yet and is currently subject of further investigation. Lastly, pulmonary monocyte influx, which has been associated with reduced SP production in other pulmonary disorders, might also result in the production of mediators that influence alveolar integrity (61,62).

Figure 1 illustrates our current understanding of HPS pathogenesis.

Human studies in HPS

Despite significant progress in fundamental HPS research, effective pharmacological therapies are currently lacking. Small human studies reporting on the use of L-NAME, norfloxacin and pentoxifylline, agents that have been proven effective in experimental HPS, had contradictory results (Table 2) (63–70). Other drugs, including mycophenolate mofetil, paroxetine, iloprost, somatostatin, almitrine and cyclooxygenase inhibitors, have been empirically tried in small studies and also did not show a clear benefit (71). Improvement of gas exchange was documented in three studies in which HPS patients were treated with garlic, which has been reported to have anti-angiogenic activity and is known to alter the gut microbiome, two components that are implicated in experimental HPS pathogenesis (72–74). However, it is difficult to draw clinical conclusions from the small number of included patients. To fully assess new therapies, larger multi-center clinical trials will be needed.

Potential new therapeutic strategies in HPS

Table 3 gives an overview of potential interventions for therapy, derived from findings in experimental HPS, grouped based on risk to benefit ratio. Angiogenesis inhibition and gut decontamination are relatively low risk strategies with potential benefit. Tyrosine kinase inhibitors are potent anti-angiogenic agents and have a track record of chronic use in patients with cirrhosis and HCC, where reductions in portosystemic shunts have been observed (75). One multi-center pilot RCT assessing the effects of sorafenib in HPS has just completed enrollment (NCT02021929). Although generally well-tolerated, tyrosine kinase inhibitor toxicity may be significant and they are not approved in Child Pugh class C patients. Anti-PIGF antibodies, which selectively inhibit pathological vessel formation, are anticipated to have less off-target toxicity and may be a more attractive therapeutic strategy. Intestinal decontamination, aimed at decreasing endotoxemia, might reduce pulmonary inflammation and may be beneficial. In one human study, norfloxacin failed to improve gas exchange (67). However, only 10 HPS patients were included and the treatment duration was limited to 4

weeks (67). Since antibiotics would not be anticipated to completely eliminate endotoxemia, long-term administration would likely be required.

Therapies with potential benefit but intermediate risk include ET receptor antagonists to modulate pulmonary vascular tone and caspase inhibitors to block AT2 cell apoptosis. In experimental studies, selective ET_B receptor inhibition significantly improves HPS, although vasoconstrictor effects would be anticipated in other vascular beds and potentially compromise function. Thus, ET_B receptor antagonists have not been developed for human use. Dual ET receptor antagonists are in use in cirrhosis for the treatment of portopulmonary hypertension, although in this situation it is not defined whether the opposing vasoconstrictive and vasodilatory actions of the receptor types would lead to improvement in the setting of HPS. Moreover, mixed ET receptor antagonists may have hepatic toxicity (76) ([LiverTox.nih.gov](https://livertox.nih.gov)). Caspase inhibitors have been used in therapeutic trials in hepatic fibrosis and PH, and are generally well-tolerated. However, the pulmonary effects of these agents would be anticipated to target AT2 cell alterations and not the vascular abnormalities of HPS, and are therefore unlikely to be effective as primary therapy.

TNF inhibition might have beneficial effects in HPS, but is considered high risk. In two small pilot human studies of HPS, TNF inhibition with pentoxifylline had conflicting results on gas exchange (68–70). In one of these studies, pentoxifylline was poorly tolerated (70). Currently available potent biologic TNF inhibitors are generally avoided in decompensated liver diseases as they may cause hepatotoxicity, increase risk of infection and may be associated with reactivation of hepatitis B (77).

Finally, a number of agents are considered to have low or unknown risk, with uncertain potential benefit. For example, modulating the circulating bile acid pool or supplementing surfactant could mitigate AT2 cell dysfunction. However, such a strategy, similar to caspase inhibition, would be anticipated to have a limited impact on the vascular alterations in HPS. Pharmacological inhibition of the chemokine receptors CX3CR1 and CXCR2 could inhibit intravascular monocyte accumulation and improve HPS. Such an approach has parallels in ongoing trials of chemokine receptor antagonists in human liver disease (NCT02217475, NCT0265362, NCT03059446, NCT03028740). Challenges include the high degree of redundancy in chemokine networks and the lack of clinically available CX3CR1 and CXCR2 antagonists.

Based on the number of potential mechanisms of HPS, it is possible that targeting multiple pathways may be needed in future trials.

Conclusion

Over the past several decades, the clinical importance of liver-lung interactions in chronic liver disease, and specifically the entity of HPS, has been widely appreciated. The CBDL experimental model has provided a pathogenic framework to explore mechanisms of human disease. Impaired gas exchange in experimental HPS involves both vascular (perfusion) and alveolar (ventilation) defects, and is driven by ET-1-mediated endothelial dysfunction, endotoxemia with subsequent intrapulmonary monocyte recruitment, pathological

angiogenesis, and AT2 cell dysfunction. Future priorities are to identify links between experimental and human disease and to organize multicenter RCTs, trialing individual and combined treatments, directed at pathophysiological targets identified in preclinical studies.

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Abbreviations

HPS	hepatopulmonary syndrome
IPVDs	intrapulmonary vascular dilations
CBDL	common bile duct ligation
TAA	thioacetamide
PH	portal hypertension
PPVL	partial portal vein ligation
AT2	alveolar type II
NO	nitric oxide
L-NAME	N-nitroarginine methyl ester
eNOS	endothelial nitric oxide synthase
iNOS	inducible nitric oxide synthase
GC	guanylate cyclase
IV	intravenous
ET-1	endothelin-1
ET_A	endothelin A
ET_B	endothelin B
HO-1	heme oxygenase 1
CO	carbon monoxide
TNFα	tumor necrosis factor alpha
CX3CL1	fraktalkine
CX3CR1	fraktalkine receptor
MCP-1	monocyte chemoattractant protein 1

ENG	endoglin
vWF	von Willebrand Factor
VCAM-1	vascular cellular adhesion molecule 1
VEGF-A	vascular endothelial growth factor A
PDGF	platelet-derived growth factor
PIGF	placental growth factor
VEGFR1	vascular endothelial growth factor 1
VEGFR2	vascular endothelial growth factor receptor 2
RCT	randomized controlled trial
FEV1	forced expiratory volume in the first second
FVC	forced vital capacity
SP	surfactant protein

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Figure 1. Current understanding of HPS pathogenesis

Salient features of experimental HPS induced by CBDL include pulmonary ET-1-induced endothelial dysfunction, monocyte infiltration, angiogenesis and AT2 cell dysfunction. These overlapping events drive the development of hypoxemia and represent potential therapeutic targets.

Abbreviations: CBDL, common bile duct ligation; TNFα, tumor necrosis factor alpha; HO-1, heme oxygenase-1; iNOS, inducible nitric oxide; CO, carbon monoxide; NO, nitric oxide; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; CX₃CR1, fraktalkine receptor; CX₃CL1, fraktalkine; ET-1, endothelin-1; ET-1_BR, endothelin B receptor; eNOS, endothelial nitric oxide synthase; PDGFR, platelet-derived growth factor receptor; VEGFR1 and 2, vascular endothelial growth factor receptors 1 and 2; AT2, alveolar type II.

Table 1

Overview of preclinical studies in hepatopulmonary syndrome

Target and compound	Mechanism of action	Animal species	Route	Intervention point	Effect on lungs	Effect on liver and hemodynamics	Ref
Intrapulmonary vascular dilations							
<i>Vascular tone</i> L-NAME	NOS inhibitor	Rats	O	Preventive	↓ intrapulmonary shunting ↑ gas exchange ↓ exhaled NO	↓ CI ↑ SVR	14
Tin protoporphyrin	HO inhibitor	Rats	IP	Therapeutic	↑ eNOS, iNOS ↓ intrapulmonary shunting ↑ gas exchange ↓ eNOS ↑ iNOS, HO-1	= PVP = MAP = spleen weight	26
<i>ET-1-induced endothelial dysfunction</i> BQ788	ET _B receptor antagonist	Rats	IV	Therapeutic	↓ intrapulmonary shunting ↑ gas exchange ↓ eNOS ↓ ED1, HO-1, iNOS ↓ CX3CL1, CX3CR1 ↓ microvessel density	= PVP = MAP	9, 41
<i>Bacterial translocation, endotoxemia, monocyte infiltration, chemotaxis</i> Norfloxacin	Quinolone, active against Gram-negatives	Rats	OG	Preventive	↓ intrapulmonary shunting ↑ gas exchange ↓ eNOS ↓ macrophages, iNOS	↓ CI	29
Pentoxifylline	Phosphodiesterase inhibitor, blocks TNF α synthesis	Rats	OG	Preventive	↓ intrapulmonary shunting ↑ gas exchange ↓ phagocytic capacity ↓ iNOS	= PVP = spleen weight ↓ CI ↑ SVR	25

Target and compound	Mechanism of action	Animal species	Route	Intervention point	Effect on lungs	Effect on liver and hemodynamics	Ref
			O	Therapeutic	↓ intrapulmonary shunting ↑ gas exchange ↓ ET _A receptor, eNOS ↓ ED1, HO-1, iNOS ↓ microvessel density, vWF, PCNA	= MAP = PVP	36, 45
Anti-TNF α Ab	TNF α neutralization	Rats	IP	Therapeutic	↓ VEGF-A, VEGFR2 ↓ CXCR2		39
Liposomal clodronate	Macrophage depletion	Rats	IV	Therapeutic	↓ intrapulmonary shunting ↑ gas exchange ↓ iNOS ↑ gas exchange ↓ ED1 ↓ microvessel density, vWF, PCNA	↑ MAP ↓ CO ↑ SVR ↓ CO	37 27
Gadolinium chloride	Macrophage depletion	Rats	IV	Preventive	↑ gas exchange ↓ ED1 ↓ microvessel density, vWF, PCNA	↓ CO	27
Anti-CX3CR1 Ab	CX3CR1 neutralization	Rats	IP	Therapeutic	↑ gas exchange ↓ microvessel density, vWF, PCNA ↓ VEGF-A, VEGFR2 ↓ ED1	= PVP = spleen weight	38, 41
SB225002	CXCR2 inhibitor	Rats	IP	Therapeutic	↑ gas exchange ↓ ED1 ↓ microvessel density, vWF	= PVP = spleen weight = MAP = liver fibrosis	39

Intrapulmonary arteriovenous shunt formation

rAAV E+A	Broad-spectrum angiogenesis inhibitor	Rats	IM	Preventive	↑ gas exchange	= PVP	45
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Target and compound	Mechanism of action	Animal species	Route	Intervention point	Effect on lungs	Effect on liver and hemodynamics	Ref
Sorafenib	VEGFR, PDGFR, Raf inhibitor	Rats	OG	Therapeutic	↓ microvessel density, vWF ↓ VEGF-A, VEGFR2 ↓ ED1 ↓ intrapulmonary shunting ↑ gas exchange ↓ eNOS ↓ ED1 ↓ VEGF-A, VEGFR2 ↓ microvessel density, vWF	= MSAP ↓ PVP ↓ αSMA ↓ spleen weight ↓ CK-19 ↓ PCNA ↓ VEGF-A ↓ ET-1	47, 48
Anti-PIGF Ab	PIGF neutralization	Mice	IP	Preventive and therapeutic	↓ intrapulmonary shunting ↑ gas exchange ↓ microvessel density, vWF, ENG, VCAM-1 ↓ MCP-1 ↓ ED1	= liver fibrosis = spleen weight = MCP-1	7
Respiratory epithelium							
AT2 cell loss Z-DEVD-FMK	caspase 3 inhibition	Rats	IP	Therapeutic	↑ gas exchange ↓ cell death ↓ microvessel density, vWF	Not assessed	59

Abbreviations: L-NAME, N-nitroarginine methyl ester; NOS, nitric oxide synthase; O, orally; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; CI, cardiac index; SVR, systemic vascular resistance; ETB, endothelin B; IV, intravenously; HO-1, hemeoxygenase 1; CX3CL1, fractalkine; CX3CR1, fractalkine receptor; PVP, portal venous pressure; MAP, mean arterial pressure; IP, intraperitoneally; OG, oral gavage; TNFα, tumor necrosis factor alpha; vWF von Willebrand Factor; PCNA, proliferating cell nuclear antigen; VEGF-A, vascular endothelial growth factor A; VEGFR2 vascular endothelial growth factor receptor 2; CXCR2, interleukin 8 receptor beta; CO, cardiac output; rAAV E+A, recombinant adeno-associated virus-6 encoding human endostatin plus angiotatin; IM, intramuscularly; PDGFR, platelet-derived growth factor receptor; αSMA, alpha smooth muscle actin; CK-19, cytokeratin 19; ET-1, endothelin-1; PIGF, placental growth factor; ENG, endoglin; VCAM-1, vascular cellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; AT2, alveolar type II.

Table 2

Overview of human studies trialing preclinically tested medical therapies for the treatment of hepatopulmonary syndrome

Study (reference)	Patients	Drug	Dose	Duration	Clinical outcome
Intrapulmonary vascular dilations					
<i>Vascular tone</i>					
Maniscalco <i>et al.</i> (63)	1 patient with cryptogenic cirrhosis	L-NAME	8 mg/kg IV	5 minutes	no improvement of clinical symptoms or gas exchange
Gómez <i>et al.</i> (64)	10 cirrhotic patients	L-NAME	162 mg in 4 mL saline	Neb over 12 minutes	no change in shunting or gas exchange
Brussino <i>et al.</i> (65)	1 patient with HCV cirrhosis	L-NAME	99.5 mg in 3 mL saline	Neb	improvement of clinical symptoms, shunting and gas exchange
<i>Bacterial translocation, endotoxemia, pulmonary monocyte infiltration, chemotaxis</i>					
Añel and Sheagren (66)	1 patient with cirrhosis	Norflaxacin	400 mg 2x/day	4 weeks	improvement of clinical symptoms and gas exchange
Gupta <i>et al.</i> (67)	11 patients with cirrhosis	Norflaxacin	400 mg 4x/day	1 month	no improvement of gas exchange
Gupta <i>et al.</i> (68)	9 patients with cirrhosis	PTX	400 mg 3x/day	3 months	improvement of clinical symptoms and gas exchange
Kianifar <i>et al.</i> (69)	10 pediatric patients with cirrhosis	PTX	20 mg/kg/day	3 months	improvement of gas exchange
Tanikella <i>et al.</i> (70)	9 patients with cirrhosis	PTX	400 mg 1x-2x-3x/day	7-7-42 days	no improvement of gas exchange
Intrapulmonary arteriovenous shunt formation					
ClinicalTrials.gov NCT02021929	30 Child-Pugh A–B patients	Sorafenib	400 mg 1x/day	3 months	? gas exchange ? shunting ? biomarker levels

Abbreviations: L-NAME, N^G-nitro-L-arginine methyl ester; PTX, pentoxifylline; IV, intravenously; Neb, nebulized.

Table 3

Potential new treatments for hepatopulmonary syndrome aimed at pathophysiological targets

Intervention	Studies	Expected effect	Advantages	Disadvantages
Potential benefit – Low risk				
VEGF(R) and PDGF(R) inhibitors	Animal	↓ angiogenesis	Experience in cirrhosis	Potential toxicity and drug resistance
PIGF inhibitors	Animal	↓ pathological angiogenesis	Experience in cancer, minimal side effects	Limited human data
Antibiotics	Human + animal	↓ pulmonary inflammation	Experience in cirrhosis, few side effects	Limited studies with negative results
Potential benefit – Intermediate risk				
ET receptor antagonists	Animal	↓ vasodilation	Experience in Child Pugh A/B, few side effects	Uncertain effect due to dual ET _{A+B} inhibition
Caspase inhibitors	Animal	↓ AT2 cell death	Experience in cirrhosis, few side effects	No effect on IPVDs or shunts
Potential benefit – High risk				
TNFα inhibitors	Human + animal	↓ pulmonary inflammation	Experience in cirrhosis (pentoxifylline)	High risk of side effects (pentoxifylline) Limited studies with conflicting results (pentoxifylline) Hepatotoxicity, infection risk, hepatitis B reactivation (biologicals)
Uncertain potential benefit – Low or unknown risk				
Bile acid modulation	N/A	↓ AT2 cell dysfunction	Experience in cirrhosis, few side effects	Limited data, no effect on IPVDs or shunts
Surfactant supplementation	N/A	↓ AT2 cell dysfunction	Few side effects	Limited data, no effect on IPVDs or shunts
Chemokine (receptor) antagonists	Animal	↓ pulmonary inflammation	Early experience in cirrhosis, few side effects	Limited experience, redundancy of chemokine network

Abbreviations: VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PIGF, placental growth factor; ET, endothelin; TNFα, tumor necrosis factor alpha; AT2, alveolar type II; PH, portal hypertension; IPVDs, intrapulmonary vascular dilations.