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Original Article

# The effects of pioglitazone in cirrhotic rats with hepatopulmonary syndrome

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**Abstract**

*Background*: Hepatopulmonary syndrome (HPS) is characterized by oxygen desaturation and increased intrapulmonary shunting formation in cirrhosis. Due to an unclarified mechanism, there is still no effective therapy except liver transplantation. Recent studies revealed that pulmonary angiogenesis may participate in pathogenesis, in which nitric oxide (NO) and vascular endothelial growth factor (VEGF) play roles. Pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, exerts anti-angiogenesis effect. However, whether pioglitazone influences pulmonary angiogenesis, shunting and HPS remains unexplored.

*Methods*: Cirrhosis with HPS was induced in Spraque-Dawley rats with common bile duct ligation (CBDL). Pioglitazone (10 mg/kg/day, oral gavage) or vehicle was administered from 8th to 28th day post CBDL. On the 28th day, the mortality rate, hemodynamic parameters, concentrations of plasma glucose and liver biochemistry parameters, and arterial blood gas data were evaluated. Lungs were dissected for protein expression analyses. In another series, intrapulmonary shunting degree was determined by color microsphere method in paralleled groups.

*Results*: The survival rates were similar in HPS rats with or without pioglitazone administration. Pioglitazone did not influence the hemodynamic parameters, glucose and liver biochemistry levels, oxygen saturation and alveolar arterial gradient, but significantly down-regulated pulmonary VEGF protein expression, endothelial NO synthase (eNOS) activation, and decreased intrapulmonary shunts. Pioglitazone significantly decreased intrapulmonary shunts as compared with the vehicle ($18.1 \pm 4.5$ vs. $9.8 \pm 3.6$, $p = 0.015$).

*Conclusion*: Pioglitazone down-regulated VEGF, eNOS and decreased intrapulmonary shunts without improving oxygenation. The current finding suggests a multifactorial mechanism of HPS that could not be successfully overcome merely by pioglitazone-induced anti-angiogenesis and shunting reduction.



*Keywords:* Angiogenesis; Hepatopulmonary syndrome; Liver cirrhosis; Pioglitazone; Portal hypertension; Shunting

## 1. Introduction

Hepatopulmonary syndrome (HPS) is characterized by arterial oxygen desaturation in patients with chronic liver disease, which poses a dismal outcome.[1] Three main components of HPS include hypoxia with increased alveolar arterial oxygen gradient ($AaPO_2$), intrapulmonary vasodilatation and increased shunting vessels, and chronic liver disease (mostly liver cirrhosis with portal hypertension). The intrapulmonary

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vasodilatation and increased shunting are responsible for abnormal gas exchange and hypoxia in HPS.[2,3] No effective therapy has been documented except liver transplantation.[4] However, emerging evidence on the pathogenesis of HPS has suggested the possibility of medical treatment.[5,6] One piece of that evidence, angiogenesis has just recently been identified.[5] Zhang et al. demonstrated that HPS was associated with pulmonary angiogenesis and vascular endothelial growth factor (VEGF) production.[5] In animals with CBDL-induced HPS, vascular VEGF synthesis and activation of VEGF-dependent signaling pathway increased pulmonary shunting degree. Furthermore, inhibition of monocyte accumulation by pentoxifylline decreased VEGF generation and was associated with reduced angiogenesis and ameliorated HPS.[5] We found that sorafenib, a multikinase inhibitor with anti-angiogenesis effect, reduced the pulmonary shunting and improved hypoxia in CBDL rats. This action might be mediated through VEGF/VEGF receptor 2 (VEGFR2) pathway inhibition.[7]

It has also been noted that endothelin-1 (ET-1) produced by the injured liver activates pulmonary $ET_B$ receptors, resulting in nitric oxide (NO)-mediated vasodilation via endothelial NO synthase (eNOS) upregulation.[8] In agreement with this notion, $ET_B$ receptor knockout inhibited pulmonary eNOS activation and improved HPS in CBDL rats.[9] Also, increased tumor necrosis factor-α (TNF-α) related to bacterial translocation in cirrhosis led to intravascular macrophage accumulation/activation and enhanced inducible NOS (iNOS) expression in animals with experimental HPS, which was improved by TNF-α inhibition.[10]

Thiazolidinediones (TZDs) are anti-diabetic agents that improve insulin sensitivity. Due to the concern of hepatic and cardiovascular adverse effects, nowadays, only pioglitazone is approved for clinical use based upon the following findings: first, the incidence of hepatotoxicity of pioglitazone is not different from other anti-diabetic drugs[11,12]; second, meta-analysis and a clinical trial reveal that pioglitazone use is associated with a significantly lower risk of myocardial infarction and stroke.[13,14] Pioglitazone is a nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) activator.[15,16] Using a high-cholesterol fructose diet-feeding rat model, Collino et al. demonstrated that pioglitazone significantly reduced hepatic expression of TNF-α.[17] Furthermore, pioglitazone improved hepatic ischemia/reperfusion injury in rats via TNF-α suppression.[18]

It is worth noting that pioglitazone also inhibits carcinogenesis,[19] which may be due to its anti-angiogenesis and anti-inflammation properties.[20] Pioglitazone elicited partial remission in patients with advanced sarcoma and malignant vascular tumors via anti-angiogenesis.[21,22] It also inhibited corneal neovascularization, rendering it promising for the treatment of diabetic retinopathy.[23] In addition, pioglitazone appears beneficial in patients with active psoriatic arthritis due to its anti-angiogenesis and anti-inflammation effects.[24] With regard to its influences on NO, it has been reported that in patients with type 2 diabetes, pioglitazone significantly reduced the level of eNOS.[25] Pioglitazone ameliorated eNOS activity in diabetic mice as well.[26] It is noteworthy that pioglitazone decreased portosystemic shunting via modulation of splanchnic inflammation and neoangiogenesis in cirrhotic rats, which was related to splanchnic eNOS and VEGF down-regulation.[27]

Taking the aforementioned factors into consideration, this study aimed to explore if pioglitazone suppressed pulmonary angiogenesis, shunting and improved HPS in rats with CBDL-induced liver cirrhosis and HPS. The underlying mechanism was also evaluated.

## 2. Methods

### 2.1. Animal model

Male Sprague-Dawley rats weighing 240−270 g at the time of surgery were used. The rats were allowed free access to food and water then fasted for 12 h before the operation. Secondary biliary cirrhosis and HPS[5] were induced in the rats by CBDL. Under ketamine anesthesia (100 mg/kg, intramuscularly), the common bile duct was exposed through a midline abdominal incision, catheterized by a PE-10 catheter and doubly ligated with 3-0 silk. The first ligature was made below the junction of the hepatic ducts and the second ligature above the entrance of the pancreatic duct. The PE-10 catheter was then removed and the ligatures tightened, followed by section of the common bile duct between the ligatures. The incision was then closed and the animal allowed to recover. A high yield of secondary biliary cirrhosis was noted four weeks after the ligation.[28,29] To avoid coagulation defects, CBDL rats received weekly vitamin K injection (50 μg/kg intramuscularly). The procedures adhered to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985). This study was approved by the Institutional Animal Care and Use Committee of Taipei Veterans General Hospital (IACUC2013-090).

### 2.2. Study protocol

Pioglitazone (10 mg/kg/day, oral gavage) or vehicle (2 ml/day, distilled water) was administered beginning on the 8th day post CBDL for 3 weeks. Four weeks after CBDL, hemodynamic parameters were measured and blood was collected to determine the plasma levels of glucose, liver biochemistry parameters, and TNF-α. Arterial blood was withdrawn for blood gas analysis. Arterial gas exchange, represented by $AaPO_2$, was calculated as $150-(PCO_2/0.8)-PO_2$.[30] In the parallel groups of CBDL rats with or without pioglitazone treatment, color microsphere technique was applied to determine the intrapulmonary shunting degree.

### 2.3. Systemic and portal hemodynamic measurement

The right internal carotid artery was cannulated with a PE-50 catheter that was connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA). Continuous recordings of mean arterial pressure (MAP) and heart rate (HR) were performed on a multi-channel recorder (model RS 3400, Gould, Inc., Cupertino, CA, USA). The external zero reference was placed at the level of the mid-portion of the rat. The

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