Exendin-4 improves cardiovascular function and survival in flow-induced pulmonary hypertension

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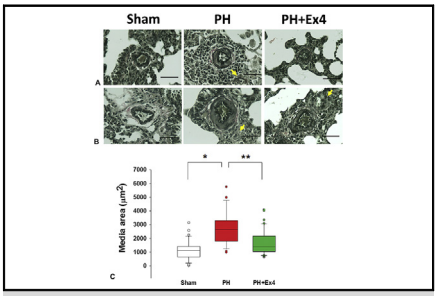
ABSTRACT

Objectives: Systemic left-to-right shunting causes pulmonary arteriopathy, leading to progressive cardiopulmonary failure and a poor prognosis. In this study, we examined the extraglycemic effect of a synthetic glucagon-like peptide, exendin-4, on pulmonary arteriopathy regression and cardiopulmonary function in nondiabetic rats.

Methods: Pulmonary hypertension (PH) was induced by monocrotaline (60 mg/kg, subcutaneous) injection followed by the creation of an aortocaval fistula. After 4 weeks, exendin-4 (1 μg/kg/day) was administered intraperitoneally for 3 consecutive weeks, followed by an assessment of cardiopulmonary function, pulmonary artery vasoreactivity, tissue and blood biochemistry, and lung histology.

Results: Exendin-4 significantly reduced right ventricle mass and pulmonary artery pressure, which improved right ventricle function and the survival rate in rats with PH. Tissue and blood interleukin-1β levels decreased, whereas pulmonary artery cyclic adenosine monophosphate levels were restored by exendin-4. Smooth muscle-myosin heavy chain-II and α-smooth muscle actin protein levels increased in the pulmonary arteries of exendin-4-treated rats. Histology showed that exendin-4 decreased the main and intra-acinar pulmonary artery medial thickness.

Conclusions: Exendin-4 treatment improved pulmonary artery function in flow-induced PH via its direct vasoactive properties, anti-inflammatory effects, and vascular smooth muscle cell phenotypic modulation. Mitigation of pulmonary arteriopathy further potentiated right ventricle performance and reduced overall mortality. These responses were associated with suppressed expression and activity of interleukin-1β and its downstream signaling molecules. Glucagon-like peptide analogs may possess pleiotropic therapeutic potential in flow-induced PH. (J Thorac Cardiovasc Surg 2017;■:1-9)



Exendin-4 attenuated pulmonary vessel hyperplasia in flow-induced pulmonary hypertension.

Central Message

Our study highlights the beneficial extraglycemic effect of exendin-4 as a potential therapeutic agent for regression of pulmonary arteriopathy secondary to flow-induced pulmonary hypertension.

Perspective

This study demonstrates that exendin-4, a glucagon-like peptide-1 analog, attenuates the progress of pulmonary arteriopathy and restores right ventricular performance, thereby reducing the overall survival of animals with flow-induced pulmonary hypertension. The pleiotropic effects of exendin-4 in pulmonary vascular remodeling may provide therapeutic potential in pulmonary hypertension secondary to increased pulmonary flow.

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BS

Pulmonary arterial hypertension (PAH) is a devastating disease that affects 15 to 60 per million people.[1] PAH is characterized by progressive remodeling of the pulmonary arterial system and the development of pulmonary arteriopathy secondary to the presence of systemic left-to-right shunts, use of pharmaceuticals, or genetic defects

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Abbreviations and Acronyms

α-SMA	= α-smooth muscle actin
cAMP	= cyclic adenosine monophosphate
c-GMP	= cyclic guanosine monophosphate
eNOS	= endothelial nitric oxide synthase
Ex4	= exendin-4
Glp-1	= glucagon-like peptide-1
IL	= interleukin
LV	= left ventricle
p	= phosphorylated
PA	= pulmonary artery
PAH	= pulmonary arterial hypertension
PH	= pulmonary hypertension
RV	= right ventricle
SM-MHC II	= smooth muscle myosin heavy chain class II
VSMC	= vascular smooth muscle cell

such as bone morphogenetic protein receptor type II mutation.[2] Approximately 30% of patients with congenital heart disease develop PAH due to excessive pulmonary blood flow[3]; their quality of life inevitably deteriorates, and long-term survival is shortened.[3,4] The survival of patients with congenital heart disease-associated PAH worsens with the development of Eisenmenger syndrome.[5] Although PAH management has improved considerably, therapeutic approaches for advanced flow-induced pulmonary hypertension (PH) are currently limited.[6]

Glucagon-like peptide 1 (Glp-1), a glucose-dependent insulinotropic peptide, belongs to the incretin family[7] and exerts its pharmacologic actions by adenylate cyclase-mediated activation of heptahelical G protein-coupled receptor.[8] In addition to its glucoregulatory effects, Glp-1 and its agonists, including exendin-4 (Ex4), have shown cardiovascular protection in experimental studies.[9] Ex4 is a Glp-1 analog that is present in the saliva of the Gila monster (*Heloderma suspectum*); it has been found to reduce neointimal hyperplasia following arterial injury.[10] It also suppresses inflammatory cytokine generation by activating adenylate cyclase and the production of cyclic adenosine monophosphate (cAMP) in monocytes and macrophages.[11] Furthermore, Ex4 attenuates hyperoxide-induced senescence in cultured vascular smooth muscle cells (VSMCs).[12] Commonly reported adverse effects of Ex4 for treatment of diabetes include nausea, vomiting, and body weight loss.[7,9] Together, we tested our hypothesis that the vascular protective and pleiotropic effects of Ex4 in modulating VSMC phenotypes and inflammatory reactions may have therapeutic potential in regressing flow-induced PH (Figure E1).

METHODS

Induction of PH in rats

All procedures were conducted in accordance with the Animal Care and Use Committee of National Cheng Kung University guidelines (approval No. 104051). Age-matched male Sprague-Dawley rats (weight, 200-250 g) were maintained on a 12-hour light-dark cycle. Single doses (60 mg/kg) of monocrotaline (Sigma-Aldrich Chemical Corp, St Louis, Missouri) were injected subcutaneously, followed by the creation of systemic left-to-right shunts that induce clinically comparable flow-induced PH.[13] Aortocaval shunts were created 7 days after monocrotaline injection, and the animals were expected to develop symptoms of right heart failure 2 to 3 weeks later.[13] Surgical procedures used to create the aortocaval shunt have been described previously (Video 1).[14,15]

Treatment Protocol

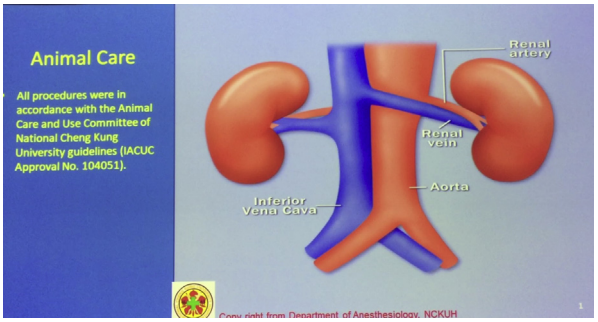
From week 4 after monocrotaline injection, flow-induced PH rats were randomly allocated to receive regular intraperitoneal injections of placebo (1 mL normal saline daily) or Ex4 (1 μg/kg/day)[16] as the PH and PH + Ex4 groups, respectively, for 3 weeks. The animals received standard chow and water ad libitum throughout the study. A total of 116 animals were included in the study analysis, and the number of animals used in each experiment is indicated in Video 1 and each figure legend. The personnel who performed hemodynamic assessments were blinded to the treatment groups.

Hemodynamic Measurements and Vasomotor Function Assessment

Echocardiography. Transthoracic echocardiography was performed on anesthetized rats immediately before euthanasia, as previously described.[15]

Invasive hemodynamic assessment. Rats were anesthetized using isoflurane (2%-3% v/v in oxygen) inhalation as previously described.[17] Rat hearts were exposed and the RV and LV were directly punctured using a 23G needle connected to a pressure transducer (Kent Scientific Corporation, Torrington, Connecticut) to measure the blood pressure.[18] Right ventricle (RV) systolic pressure was used as a surrogate indicator of pulmonary artery (PA) pressure.[19] The rats were put to death after the invasive hemodynamic measurements. The hearts and lungs were removed en bloc for analysis including lung wet-to-dry ratio.[20]

RV morphologic analysis. The rat hearts were dissected to isolate the free wall of the RV from the left ventricle (LV) and septum. The RV/LV + septum mass ratio was the index (Fulton index) of RV hypertrophy.[19]



VIDEO 1. An animated presentation of the "2-hit" model of flow-induced pulmonary hypertension shows the surgical techniques. The treatment protocol and a summary of the limitations of this animal model are also included as supplementary material. Video available at: http://www.jtcvsonline.org.