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 μ g/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-1 β 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-1 β and its downstream signaling molecules. Glucagon-like peptide analogs may possess pleiotropic therapeutic potential in flow-induced PH. (J Thorac Cardiovasc Surg 2018;155:1661-9)

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.

See Editorial Commentary page 1670.

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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

Scanning this QR code will take you to supplemental tables, figures, and video for this article.

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 flow³; 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 family⁷ 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 μ g/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/article/S0022-5223\(17\)32432-7/fulltext](http://www.jtcvsonline.org/article/S0022-5223(17)32432-7/fulltext).

Vasomotor function assessment. The main PA rings (~3 mm long) were mounted in organ chambers containing 25 mL Krebs solution, and the vasomotor function of PA was assessed by isometric tension recordings.¹⁵

Quantitative Analysis of Serum Inflammatory Markers and Blood Chemistry

Blood samples were collected before the rats were put to death. Serum interleukin (IL)-1 α , IL-1 β , IL-2, IL-6, monocyte chemoattractant protein-1, and tumor necrosis factor- α concentrations were quantified using sandwich cytokine-specific enzyme-linked immunosorbent assay detection kits (eBioscience, Frankfurt, Germany). Blood glucose, triglyceride, cholesterol, and creatinine levels were analyzed using colorimetric assays (ADVIA 1800 Chemistry System; Siemens, Munich, Germany).²¹

Immunoblotting. The proteins extracted from PA tissues (50 μ g) were prepared for incubation with appropriate primary antibodies, as previously described.^{15,22}

Histology of PAs and arterioles. The PA and lung tissue samples were fixed in 4% paraformaldehyde, paraffin wax-embedded, and cut into 5- μ m sections, which were stained with hematoxylin and eosin and Verhoeff stains. The slides were analyzed under light microscopy by observers who were blinded to the treatment groups. The intra-acinar pulmonary arterioles were defined as vessels with diameters < 150 μ m.²³ Twenty arterioles or slices of arteries were measured per animal under 400 $\times$ magnification to determine the medial thickness.

Measurement of tissue cAMP and cyclic guanosine monophosphate. PA tissue segments were incubated in Earle's salts solution as previously described.²² Tissue cyclic guanosine monophosphate (cGMP) and cAMP concentrations were determined by enzyme-linked immunosorbent assay (R&D System, Minneapolis, Minnesota).

Immunohistochemistry. Immunohistochemistry was performed for the above-described tissue sections using a Bond-Max Automated IHC stainer (Leica Biosystems Newcastle Ltd, Melbourne, Australia). The sections were incubated with rabbit anti-IL-1 β (1:200 dilution) at 25°C for 30 minutes and counterstained with hematoxylin. Quantification was performed using ImageJ software (National Institutes of Health, Rockville, Md).

Statistical Analysis

The results are presented as the mean $\pm$ standard deviation or median with 25th to 75th percentiles, as appropriate. Differences in survival rates were analyzed using the χ^2 and the log-rank tests for Kaplan-Meier curves. Intergroup differences were analyzed using the Student *t* test or 1-way analysis of variance followed by appropriate post hoc tests for multiple comparisons. The concentration curves in the vasomotor function analysis were analyzed using 2-way repeated measures analysis of variance with post hoc Holm-Sidak tests. The post hoc Tukey or Dunn test was used as appropriate for nonparametric analysis.

RESULTS

Ex4 Suppressed Serum Inflammatory Cytokines and Improved Survival

No significant changes were observed in body weight or in blood glucose, triglyceride, total cholesterol, and creatinine levels in the Ex4-treated animals (Table E1). Ex4 significantly reduced serum IL-1 α and IL-1 β concentrations in PH rats (Table 1). During the 3-week treatment, Ex4 significantly improved the survival rate of rats with flow-induced PH (77.8% vs 25.0% for PH + Ex4 vs PH on treatment day 21; *P* = .006) (Figure 1, A). The RV/LV + septum ratio was restored,

TABLE 1. Serum inflammatory markers in rats with flow-induced pulmonary hypertension (PH)

Inflammatory marker	PH	PH + Ex4	<i>P</i> value
IL-1 α (ng/mL)	44.08 $\pm$ 9.54	11.58 $\pm$ 3.04	.005
IL-1 β (pg/mL)	203.60 $\pm$ 55.92	34.13 $\pm$ 13.55	.019
IL-2 (ng/mL)	28.35 $\pm$ 4.48	26.85 $\pm$ 6.43	.838
IL-6 (pg/mL)	21.32 $\pm$ 1.75	29.03 $\pm$ 10.45	.981
MCP-1 (pg/mL)	285.71 $\pm$ 10.89	330.35 $\pm$ 40.42	.421
TNF- α (pg/mL)	2.37 $\pm$ 0.19	1.82 $\pm$ 0.14	.067

Values are presented as mean $\pm$ standard error of the mean (*n* = 6/group). Data from the sham group are not shown due to low serum titers. PH, Pulmonary hypertension; Ex4, exendin-4; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF- α , tumor necrosis factor- α .

and lung wet-to-dry ratio was reduced (Figure 1, B and C). The major cause of death was heart failure.

Ex4 Improved RV Function

Ex4 significantly reduced the PA diameter and enhanced the peak flow velocity across the RV outflow track (Table 2 and Figure E2). The RV diameter and tricuspid peak flow were restored in PH + Ex4 rats (Table 2). Ex4 treatment also significantly reduced RV systolic pressure and adjusted RV systolic pressure (RV systolic pressure_{peak}-to-LV systolic pressure_{peak} ratio) (Table E2).²⁴ The LV ejection fraction was unaffected by Ex4 treatment (*P* = .417) (Table 2). Additionally, the LV systolic and diastolic pressures were augmented in the PH + Ex4 group (Table E2).

Ex4 Potentiated the Vasomotor Function of PAs

Isometric contractions were significantly increased in response to potassium chloride (40 mM) and phenylephrine (10⁻⁹-10⁻⁵ M) in the PA of flow-induced PH rats (Figure 2, A and B), whereas Ex4 treatment significantly attenuated the potassium chloride- and phenylephrine-induced contractions (Figure 2, A and B). Endothelial-dependent relaxation in response to acetylcholine was significantly impaired in the PA of PH rats, and the relaxation curve was potentiated in Ex4-treated rats (Figure 2, C). Endothelial-independent relaxations were shifted to the right in rats with flow-induced PH but were not potentiated by Ex4 (Figure 2, D).

Ex4 Enhanced cAMP and cGMP Biosynthesis in the PAs

The high pulmonary blood flow marginally increased endothelial nitric oxide synthase (eNOS) protein levels in the PAs (*P* = .059) (Figure 3, A and B), whereas phosphorylated (p)-eNOS expression significantly increased (Figure 3, C). Ex4 did not affect the protein levels of eNOS, p-eNOS, or heat shock protein in PAs exposed to chronic high blood flow (Figure 3, A-D); however,

FIGURE 1. Cardiopulmonary effects and survival of rats with flow-induced pulmonary hypertension (PH). A, Survival outcomes with 95% confidence intervals of PH rats treated with and without Exendin-4 (Ex4) for 3 weeks; n = 16 and 18 for PH and PH + Ex4 groups, respectively. B, Right ventricle (RV) weight adjusted to weight of the left ventricle (LV) with septum (Fulton index); n = 6 for both groups. C, Lung wet:dry ratio on the day of euthanasia; n = 6/both groups. The dashed line in B and C indicates sham group. * $P < .001$ using the log-rank test. ** $P = .031$. # $P = .037$.

Ex4 significantly increased both cGMP and cAMP concentrations in the PA of PH rats ($P < .001$ and $P = .007$, respectively) (Figure 3, E and F).

Ex4 Suppressed IL-1 β and Associated Proteins in the PAs

Caspase-1 (an interleukin-converting enzyme), IL-1 β , and its downstream signaling proteins (p-P38 mitogen-activated protein kinase, total-extracellular signal-regulated kinase 1/2, and p-extracellular signal-regulated kinase 1/2)

were significantly upregulated in the PA of rats with flow-induced PH (Figure E3). Furthermore, Ex4 suppressed the abundance of caspase-1 and IL-1 β -associated proteins in the PA (Figure E3).

Ex4 Modulated VSMC Phenotypes in PAs

The abundance of smooth muscle myosin heavy chain class II (SM-MHC II) and α -smooth muscle actin (α -SMA) decreased in the flow-induced PH animals (Figure 4), whereas Ex4 restored the abundance of these

TABLE 2. Echocardiographic measurements

Variable	Sham	PH	PH + Ex4	P value
Main pulmonary artery diameter (mm)	2.87 $\pm$ 0.20	3.76 $\pm$ 0.38*	2.71 $\pm$ 0.15	.009
Pulmonary artery peak flow velocity (cm/s)	74.23 $\pm$ 7.00	59.42 $\pm$ 6.09†	82.16 $\pm$ 6.45	.046
Right ventricle diameter (mm)	4.08 $\pm$ 0.09	5.49 $\pm$ 0.34‡	4.19 $\pm$ 0.40	.001
Tricuspid peak flow velocity (cm/s)	68.85 $\pm$ 4.45	102.78 $\pm$ 9.52§	82.44 $\pm$ 5.54	.010
Left ventricle ejection fraction (%)	68.75 $\pm$ 3.47	65.91 $\pm$ 3.60	72.31 $\pm$ 3.17	.417

Values are presented as mean $\pm$ standard error of the mean (n = 6/group). PH, Pulmonary hypertension; Ex4 = exendin-4. * $P < .05$ versus other groups using the post hoc Tukey method. † $P < .05$ versus PH + Ex4 with the post hoc Dunn's method. ‡ $P < .05$ versus other groups using the post hoc Tukey method. § $P < .05$ versus sham using the post hoc Tukey method.

FIGURE 2. Vasomotor function analysis of pulmonary arteries (PAs) from rats with pulmonary hypertension (PH) with or without exendin-4 (Ex4) treatment. A, Contractile responses to 40 mM potassium chloride (KCl) and phenylephrine (PE) (10^{-5} M). B, Contractile responses of the PA to cumulative addition of PE (10^{-9} – 10^{-5} M). C, Relaxation responses to cumulative addition of acetylcholine. D, Relaxation responses to cumulative addition of diethylammonium (Z)-1-(N, N-diethylamino) diazen-1-ium-1, 2-diolate (DEA-NONOate)/nitric oxide. Relaxation was obtained during contraction using an effective concentration that achieved 60% maximum contraction of PE. Data were analyzed using analysis of variance and are presented as mean $\pm$ standard deviation; $n = 6$ to 8 rats/group. * $P = .004$ for PH versus PH + Ex4 and sham. ** $P = .017$ for PH versus PH + Ex4 and sham. *** $P < .001$. ^ $P < .001$ for PH versus PH + Ex4 and sham. # $P < .05$ for PH versus PH + Ex4 and sham. ## $P < .05$ for PH versus PH + Ex4 and sham.

proteins (Figure 4, B and C). Tissue matrix metalloproteinase-2 levels were reduced in the PH + Ex4 group (Figure 4, D).

Ex4 Attenuated the Medial Thickness of PA and Intra-acinar Arterioles

Medial thickness was significantly increased in the main PA and intra-acinar arterioles of rats with flow-induced PH (Figure E4, A and Figure 5, respectively). Three weeks of Ex4 treatment reduced the medial thickness in both the main PA (Figure E4, A) and intra-acinar arterioles (Figure 5). Plexiform arteriopathy was identified around intra-acinar arterioles with or

without Ex4 treatment (Figure 5, A and B). IHC demonstrated increased IL-1 β expression in the remodeled main PA of PH rats, and this effect was mitigated by Ex4 (Figure E4).

DISCUSSION

Although our previous study suggested that the creation of an aortocaval fistula induces medial hypertrophy and hypertension in the PA, the clinical severity, particularly the survival of the animals, was not remarkable.²² van Albada and colleagues¹³ developed a more clinically relevant model of PH by injecting a single dose of monocrotaline before creating a large systemic left-to-

FIGURE 3. Immunoblotting and enzyme-linked immunosorbent assay of pulmonary artery tissue. A, Representative proteins associated with nitric oxide production. Quantification of western blotting for (B) Endothelial nitric oxide synthase (*eNOS*) ($P = .059$). C, phosphorylated (p)-*eNOS*. D, Heat shock protein (*hsp*) 90 ($P = .543$); $n = 8$ to 10/group. E and F, Tissue concentrations of cyclic guanosine monophosphate (*cGMP*) and cyclic adenosine monophosphate (*cAMP*) in pulmonary artery homogenates after a 30-minute incubation with 3-isobutyl-1-methylxanthine (10^{-3} M). * $P < .05$. ** $P < .05$ for pulmonary hypertension (PH) versus sham and PH + endothelin-4 (*Ex4*), respectively; $n = 8$ to 10/group with 2 replicates. # $P < .001$. ## $P < .001$.

right shunt in rats. Monocrotaline, a toxic pyrrolizidine alkaloid extracted from the plant *Crotalaria spectabilis*, has been used since the 1960s to induce PH in laboratories.²⁵ Injection of monocrotaline induces a prominent inflammatory reaction, which is an important component of the pathogenesis of patients with PAH.^{26,27} The presence of the left-to-right shunt, pulmonary plexogenic arteriopathy, and the subsequent right heart remodeling in our model suggest the potential

for clinical inferences to diseases with left-to-right shunt.^{25,28} Our present findings confirm that this modification significantly enhances the mortality and cardiopulmonary dysfunction of rats. Simultaneous injection of monocrotaline and creation of aortocaval shunts remodeled the RV and PA, and mortality increased significantly 2 weeks after PH induction.

Increased pulmonary blood flow enhances *eNOS* and *p-eNOS* expression and reduces *cGMP* (nitric oxide second

FIGURE 4. Exendin-4 (*Ex4*) attenuated de-differentiation of vascular smooth muscle cells in pulmonary arteries from rats with flow-induced pulmonary hypertension (*PH*). A, Representative immunoblots of smooth muscle myosin heavy chain (*SM-MHC*) class II, α -smooth muscle actin (*SMA*), and matrix metalloproteinase (*MMP*)-2. B-D, Quantitative analysis of protein levels. $n = 5$ to 6/group. * $P < .05$. ** $P = .004$. *** $P = .001$. # $P < .05$. ## $P = .004$. ### $P = .025$.

messenger) levels in the PA.²² Ex4 enhances eNOS and nitric oxide biosynthesis in cultured endothelial cells by activating Glp-1 receptor-dependent protein kinase A-phosphoinositide-3 kinase (PKA-PI3K)/Akt-eNOS pathways.²⁹ However, excessive nitric oxide may trigger nitric oxide-cGMP-independent pathways to increase oxidative stress.³⁰ The impaired endothelial-independent relaxation responses in the PA from Ex4-treated rats indicate the presence of adverse effects of nitric oxide in our model. In our study, eNOS/p-eNOS protein levels were unaffected by Ex4, whereas the coupling activity of eNOS was improved, as supported by the restoration of cGMP tissue levels. Consequently, endothelial function and pulmonary hemodynamic measures were improved in the Ex4-treated rats.

IL-1 β is associated with primary and secondary PH.^{26,31} In our study, the serum and PA tissue contents of caspase-1 and IL-1 β -associated signaling molecules were significantly increased in PH rats. Ex4 treatment significantly downregulated IL-1 β levels in circulation and the pulmonary artery. IL-1 β downregulates cAMP in PA VSMCs by downregulating adenylyl cyclase in the p38 mitogen-activated protein kinase-dependent pathway,³²

which supports our finding of increased tissue cAMP levels in the PA of Ex4-treated PH rats. Accordingly, our study demonstrates that IL-1 β may play an important role in flow-induced pulmonary arteriopathy, which was attenuated by systemic administration of Ex4.

Next, we examined the phenotypic modulation of PA VSMCs. In the de-differentiated state, VSMCs re-enter the cell cycle.³³ The PA of flow-induced PH rats showed reduced expression of α -SMA and SM-MHC-II, suggesting that VSMCs de-differentiated from the contractile to the synthetic phenotype in response to the increased blood flow in PH.³⁴ De-differentiation of VSMCs is essential for vascular remodeling and enlargement of the internal diameter of blood vessels, which normalizes the increase in shear stress.¹⁴ In this study, Ex4 treatment clearly restored pulmonary VSMCs to the contractile phenotype with enhanced α -SMA and SM-MHC-II expression. Moreover, the increased cGMP/cAMP concentration in Ex4-treated rats may contribute to the contractile phenotypic switch of VSMCs in the remodeled PA.³⁵

Our study has several limitations. The PA remodeling observed in this model is recognized as clinically relevant,

FIGURE 5. Exendin-4 (*Ex4*) attenuated medial hyperplasia of intra-acinar pulmonary vessels in rats with flow-induced pulmonary hypertension (*PH*). Verhoeff staining of pulmonary arterioles with a diameter (A) < 50 μm and (B) 50 to 100 μm. Plexiform angiopathy was identified around pathologic arterioles in *PH*-induced rats (yellow arrow). C, Quantitative analysis of intra-acinar arterioles (<100 μm). n = 6/group, ×400 magnification; scale bar, 50 μm. **P* < .05. ***P* < .05.

but time-related pathophysiologic differences from human congenital heart diseases still exist (Video 1).¹³ Furthermore, our analyses did not account for the residual impaired endothelial-independent relaxation response in the vasomotor function test. Nevertheless, our findings support that the extraglycemic therapeutic effects of Glp-1 analogs are potentially useful in the cardiovascular system.⁹

CONCLUSIONS

Our study clearly demonstrated that monocrotaline administration followed by systemic left-to-right shunt

creation induced consistent *PH* and pulmonary arteriopathy, leading to progressive RV maladaptive remodeling and increased mortality after 6 weeks. *Ex4* treatment improved vasoreactivity in the PA via direct vasoactive, anti-inflammatory, and VSMC phenotypic modulatory effects. Furthermore, the improvement of pulmonary arteriopathy potentiated RV performance and reduced overall mortality. Therefore, our study highlights the beneficial extraglycemic effect of *Ex4* as a potential therapeutic agent for regression of pulmonary arteriopathy secondary to flow-induced *PH*.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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FIGURE E1. Summarized signal pathways of exendin-4 in endothelial and vascular smooth muscle cells. AC, Adenylate cyclase; GLP-1R, glucagon like peptide-1 receptor; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; cEPC, endothelial progenitor cell; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PI3K, phosphoinositide-3 kinase; PKA, protein kinase A; eNOS, endothelial nitric oxide synthase; Epac, exchange protein directly activated by cAMP; SOD, superoxide dismutase; HO-1, heme-oxygenase-1; VSMC, vascular smooth muscle cell; cGMP, cyclic guanosine monophosphate.

FIGURE E2. Representative echocardiographic images. Yellow dotted line indicates the position of main pulmonary artery (PA) trunk. Yellow star indicates the position of right ventricle (RV). PH, Pulmonary hypertension; Ex4, exendin-4.

FIGURE E3. Exendin-4 (*Ex4*) down-regulated interleukin (IL)-1 β signaling-related proteins in pulmonary arteries (PAs). A, Representative immunoblots of caspase-1, IL-1 β , and associated signaling proteins in PAs from rats with pulmonary hypertension (*PH*) treated with or without Ex4. B-F, Quantitative data for caspase-1, IL-1 β , phosphorylated (p)-p38 mitogen-activated protein kinase (*MAPK*), total (t)-extracellular-regulated signal kinase (*ERK*) 1/2, and p-ERK1/2. n = 6/group. **P* < .05. ***P* < .05.

FIGURE E4. Histology of main pulmonary arteries (PAs) in flow-induced pulmonary hypertension (PH). A, Hematoxylin and eosin, Verhoeff, and immunohistochemical staining of interleukin (IL)-1 β . B, Quantitative analysis of IL-1 β expression in PAs. n = 4 animals per group. $\times 40$ magnification; scale bar, 50 μ m. Ex4, Exendin-4. * $P < .05$. # $P < .05$.

TABLE E1. Serum glucose and lipid profile of rats with flow-induced pulmonary hypertension (PH)

Variables	Sham	PH	PH + Ex4	P value
Body weight	295.1 ± 110.8	354.5 ± 39.5	336.7 ± 34.5	.689
Glucose AC (dL/mL)	170.7 ± 10.5	148.5 ± 6.0	152.7 ± 32.7	.070
Triglyceride (mg/dL)	48.5 ± 26.3	55.2 ± 13.4	80.9 ± 27.6	.056
Total cholesterol (mg/dL)	49.8 ± 3.9	46.7 ± 6.1	45.4 ± 12.9	.668
Creatinine (mg/dL)	0.31 ± 0.05	0.33 ± 0.02	0.35 ± 0.07	.278

Values are presented as mean ± standard deviation. n = 6 to 8/group. PH, Pulmonary hypertension; Ex4, exendin-4; Glucose AC, before meal.

TABLE E2. Invasively measured hemodynamic data

Variables	Sham	PH	PH + Ex4	P value
Heart rate (beats/min)	258.31 ± 15.77	296.31 ± 1.19	249.39 ± 26.02	.885
RVSP _{peak} (mm Hg)	21.48 ± 3.31	67.14 ± 5.75*	47.44 ± 1.89†	< .001
RV-EDP (mm Hg)	7.74 ± 1.65	10.72 ± 1.91	17.30 ± 4.70	.207
LVSP _{peak} (mm Hg)	74.78 ± 3.53	82.62 ± 1.03	98.19 ± 3.63‡	.001
LV-EDP (mm Hg)	5.82 ± 0.84	6.32 ± 0.92	11.18 ± 1.24§	.003
RVSP _{peak} /LVSP _{peak} (%)	31.00 ± 3.00	79.29 ± 3.58*	51.43 ± 3.06†	< .001

Values are presented as mean ± standard error of the mean (n = 6/group). PH, Pulmonary hypertension; Ex4, exendin-4; RVSP_{peak}, right ventricle peak systolic pressure; RV-EDP, right ventricle end-diastolic pressure; LVSP_{peak}, left ventricle peak systolic pressure; LV-EDP, left ventricle end-diastolic pressure. *P < .05 versus other groups using post hoc Tukey test. †P < .05 versus other groups using post hoc Tukey test. ‡P < .05 versus PH using post hoc Dunn's method. §P < .05 versus PH using post hoc Tukey test.