Research Article

Rosiglitazone Attenuated Endothelin-1-Induced Vasoconstriction of Pulmonary Arteries in the Rat Model of Pulmonary Arterial Hypertension via Differential Regulation of ET-1 Receptors

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Pulmonary arterial hypertension (PAH) is a fatal disease characterized by a progressive increase in pulmonary arterial pressure leading to right ventricular failure and death [1]. ET-1 plasma level was elevated in the patients and experimental models for PAH [2, 3]. Expression of ET-1 was increased in lung tissues of PAH patients, predominantly in pulmonary arteries [4, 5]. ET-1 has 2 major subtypes of receptors: ET-A receptor (ET₆R) is expressed on vascular smooth muscle cells (SMCs) and mediates vasoconstriction, whereas ET-B receptor (ET₇R) is predominantly expressed in endothelial cells (ECs), where it primarily mediates vasodilatation and the clearance of ET-1. Expression of ET₆R was upregulated in the lung tissues and pulmonary arteries from PAH patients with a well-established pathophysiological role [6–8]. However, a role of ET₇R was rather controversial with the reports of unaltered, increased, or decreased expressions in the vessel tissues from various PAH conditions [9–15].

Emerging evidence suggests that peroxisome proliferator-activated receptor-γ (PPARγ) agonists might have therapeutic role in treating PAH [16]. PPARγ regulates the transcription of genes involved in glucose and lipid metabolism, inflammation, as well as vascular remodeling [17–19]. The
expression of PPARγ was reduced in the lungs from the PAH patients and the rat models [20, 21]. Similarly, mice with deletion of PPARγ in SMCs or ECs developed PAH. Pharmacological activation of PPARγ ameliorated PAH [21–25]. In ECs, PPARγ activators inhibited thrombin- or oxidized low-density lipoproteins- (LDL-) induced ET-1 production [26, 27]. In particular, we recently observed that PPARγ agonist rosiglitazone attenuated ET-1-induced vasoconstriction through upregulation of ETBR in ECs [28]. However, whether the regulation of ETBR accounts for the ameliorative effect of PPARγ agonists in PAH arteries remains to be elucidated. In the present study, we examined the role of rosiglitazone on ET-1-induced vasocontraction of pulmonary arteries in rat PAH models and the underlying mechanism.

2. Materials and Methods

2.1. Animals, Cell Culture, and Reagents. Male Sprague-Dawley rats were used and the experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals with the approval by the institutional committee. Polyclonal rabbit anti-ETBR antibody was from Abcam. Polyclonal rabbit anti-ETAR was from Santa Cruz Biotechnology. ET-1 and \( \text{N}^\text{G} \)-nitro-\( \text{L} \)-arginine methyl ester (\( \text{L} \)-NAME) were from Sigma-Aldrich Co., rosiglitazone was from GlaxoSmithKline, and A192621 was from Abbott Laboratories.

2.2. Chronic Hypoxia Induced PAH in Rat. Rats were exposed to normobaric hypoxia (10% oxygen) or normoxia (21% oxygen) for 3 weeks and then treated with rosiglitazone (20 mg/kg per day) or water with oral gavage for 3 days.

2.3. Isometric Tension Measurement. Left lungs were removed and placed in oxygenated Krebs-Henseleit solution. Pulmonary arteries were carefully dissected from adjacent connective tissue and cut into several ring segments of \( \approx 2 \text{ mm} \) long for measuring isometric force. Organ chambers (Multi Myograph System, Danish Myo Technology A/S) were filled with (37°C) Krebs solution containing (in mmol/L) 119.0 \( \text{NaCl} \), 4.7 \( \text{KCl} \), 2.5 \( \text{CaCl}_2 \), 1.0 \( \text{MgCl}_2 \), 25.0 \( \text{NaHCO}_3 \), 1.2 \( \text{KH}_2\text{PO}_4 \), and 11.0 \( \text{D}-\text{glucose} \). The Krebs solution in the organ bath was initially open to room air, being bubbled with mixed 95% \( \text{O}_2 \) and 5% \( \text{CO}_2 \). Each ring was suspended between 2 tungsten wires (diameter, 40 \( \mu \text{m} \)) in the chamber under optimal resting tension (2.5 mN as previously determined for the pulmonary arteries) and left for 90-minute equilibration. Vasoreactivity was measured to compare contractions in response to ET-1 (1 to 50 nmol/L) in the absence and presence of \( \text{L} \)-NAME (100 \( \mu \text{mol/L} \)). The effects of antagonist of ETBR were tested on ET-1-induced contractions.

2.4. Western Blot Analysis. Pulmonary arteries were dissected, frozen in liquid nitrogen, and homogenized in RIPA lysis buffer containing protease inhibitors. Protein lysates separated on 12.5% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and transferred to PVDF membranes, which were blocked with 5% nonfat milk in Tris-buffered saline-Tween (0.2%) (TBS-T) for 1h, incubated overnight with primary antibody and then horseradish peroxidase-(HRP-) conjugated secondary antibody, and visualized with ECL reagent.

2.5. Statistical Analysis. Results represent mean ± SEM. Comparisons among groups involved ANOVA followed by
3. Results

3.1. Rosiglitazone Ameliorated ET-1-Mediated Vasoconstriction in Rats with PAH. To investigate the effect of rosiglitazone on vasoconstriction of pulmonary arteries induced by ET-1, pulmonary arteries from normoxic-, CH-, and rosiglitazone-treated CH-rats were dissected from groups of animals for isometric tension measurement responding to ET-1. The ET-1-induced contractions in pulmonary arteries were elevated in PAH rats compared to the normoxic rats. Treatment with PPAR\γ agonist rosiglitazone (20 mg/kg per day) reversed the vasocontractive effect of ET-1 (Figure 1). However, this effect of rosiglitazone was abolished by the treatment with the inhibitor of endothelial nitric oxide synthase (eNOS) L-NAME, indicating a NO-dependent mechanism (Figure 2).

3.2. Rosiglitazone Increased ET\β R Protein Levels in Pulmonary Arteries from PAH Rats. To understand the mechanism for the effect of rosiglitazone on ET-1-induced vasoconstriction in pulmonary arteries, we examined the protein level of ET\β R with Western blotting. As shown in Figure 3, ET\β R protein level was unaltered in the pulmonary arteries from CH-induced PAH rats. However, rosiglitazone treatment increased the expression of ET\β R. In contrast, it reduced the expression of ET\α R (Supplemental Figure 2 available online at http://dx.doi.org/10.1155/2014/374075).

3.3. Inhibitory Effect of Rosiglitazone Is Abolished by ET\β R Antagonist. To examine the functional role of ET\β R in mediating the rosiglitazone effect on ET-1-induced vasoconstriction, pulmonary arteries were dissected from normoxic-, CH-, and rosiglitazone-treated CH-rats to measure the ET-1-responsive isometric tension in the presence or absence of A192621, a selective ET\β R antagonist. In normoxic and PAH rats, A192621 (10 nmol/L) did not significantly alter the ET-1-induced contraction (Figures 4(a) and 4(b)). However, in the rosiglitazone-treated pulmonary arteries, A192621 abolished the ameliorative effect on the ET-1-induced vasoconstriction (Figure 4(c)).

4. Discussion

The vascular effects of ET-1 are mediated by 2 pharmacologically distinct G protein-coupled receptors, ET\α R and ET\β R [29]. ET\α R is mostly expressed in SMCs and mediates the vasoconstrictive and proliferative effects of ET-1 [30]. However, ET\β R expressed in ECs mediates endothelial-dependent vasodilation by stimulating the production of NO and prostacyclin, prevents apoptosis, and promotes the
clearance of ET-1 [31, 32]. ET\textsubscript{B}R is present in low densities on vascular smooth muscle cells where its activation induces vasoconstriction [33, 34]. Since ET\textsubscript{B}R elicits vasodilation and vasoconstriction, its vascular functions in pulmonary arterial hypertension need to be further characterized. ET\textsubscript{B}R-deficient rats developed exacerbated PAH after exposure to chronic hypoxia, characterized by elevated pulmonary arterial pressure, diminished cardiac output, increased right ventricular hypertrophy, and increased total pulmonary resistance. Plasma ET-1 level and mRNA of ET-converting enzyme-1 (ECE-1) were much higher in lungs from ET\textsubscript{B}R-deficient rats compared with control rats. In ET\textsubscript{B}R-deficient rats, the pulmonary vessels showed less endothelial NO synthase (eNOS) and NO production, supporting a role of NO in ET\textsubscript{B}R-mediated vasodilation in the pulmonary vasculature [35]. Other studies in monocrotaline (MCT) induced PAH rats also showed that ET\textsubscript{B}R deficiency accelerated the progression of PAH and neointimal lesion [36, 37]. Although both ET\textsubscript{A}R antagonist (ambrisentan) and dual ET\textsubscript{A}R/ET\textsubscript{B}R antagonist (bosentan) have been approved for treatment of PAH [38], selective antagonists for ET\textsubscript{A}R and ET\textsubscript{B}R appeared to have different effects on PAH. In a dog model for PAH, ET\textsubscript{B}R antagonist RES-701-1 was found to increase pulmonary arterial pressure whereas sarafotoxin S6c, an ET\textsubscript{B}R agonist, decreased pulmonary arterial resistance [39]. In addition, ET\textsubscript{B}R antagonist also elevated ET-1 concentrations in both in vivo and in vitro studies [40]. These findings suggest that activation of ET\textsubscript{B}R may play a protective role in the PAH.

In addition to three categories of FDA-approved treatments including prostanoids, ET-1 receptor antagonists, and phosphodiesterase 5 (PDE5) inhibitors, PPAR\textsubscript{\gamma} agonists thiazolidinediones (TZDs) including rosiglitazone and
pioglitazone have shown beneficial effects in animal models of PAH. In rodent PAH models induced by MCT or hypoxia and those associated with insulin resistance, TZDs were found to effectively reduce pulmonary arterial pressure and right ventricular hypertrophy [21, 22, 24, 25, 41]. Recently, we showed that rosiglitazone reversed pulmonary arterial remodeling and inhibited vasoconstriction in response to serotonin in the rat PAH models induced by MCT and hypoxia. Although the molecular mechanisms underlying the TZD effects on PAH development remain unclear, a generally accepted hypothesis is that TZDs may act via their receptor PPARy to modulate the expression of key genes involved in the pathogenesis of PAH such as ET-1, eNOS, p27KIP1, adiponectin, apoE, MMP, and RhoA/ROCK. In this study, we provided in vivo evidence that rosiglitazone ameliorated ET-1-induced vasocontraction in the pulmonary arteries of PAH rats (Figure 1). The ameliorative effect of rosiglitazone was mediated via differential regulation of ET-1 receptors. In particular, the upregulation of ETbR might play a major role because rosiglitazone treatment increased the expression of ETbR in the pulmonary arteries (Figure 3) and A192621, a selective antagonist of ETbR, abrogated the effect (Figure 4). Conversely, rosiglitazone inhibited the induction of ETA R in the pulmonary arteries of PAH rats (Supplemental Figure 2). It is conceivable that rosiglitazone may have the vasoprotective effects by altering the ratio of ETA R/ETbR receptors. ETbR in ECs may increase Ca2+ influx and the activation of eNOS, which leads to the production of NO and induction of vascular relaxation. This notion is corroborated with the result that the effect of rosiglitazone was abolished in the presence of L-NAME, an inhibitor of eNOS (Figure 2). Importantly, the induction of endothelial ETbR is considered to be a PPARy-specific mechanism as we previously identified ETbR to be a direct target gene of PPARy [28].

5. Conclusions

In conclusion, we demonstrated that rosiglitazone upregulated the expression of ETbR, which mediated the decreased vasoconstriction in the rat models of PAH. This finding suggested a new mechanism for the protective role of PPARy in the development of PAH.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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References

[1] H. W. Farber and J. Loscalzo, “Mechanisms of disease: pulmonary arterial hypertension,” New England Journal of Medicine, vol. 351, no. 16, pp. 1655–1665, 2004.
[2] T. Miyachi, R. Yokike, S. Sakai et al., “Contribution of endogenous endothelin-1 to the progression of cardiopulmonary alterations in rats with monocrotaline-induced pulmonary hypertension,” Circulation Research, vol. 73, no. 5, pp. 887–897, 1993.
[3] D. J. Stewart, R. D. Levy, P. Cernacek, and D. Langleben, “Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease?” Annals of Internal Medicine, vol. 114, no. 6, pp. 464–469, 1991.
[4] A. Giaid, M. Yanagisawa, D. Langleben et al., “Expression of endothelin-1 in the lungs of patients with pulmonary hypertension,” New England Journal of Medicine, vol. 328, no. 24, pp. 1732–1739, 1993.
[5] T. J. Stelzner, R. F. O’Brien, M. Yanagisawa et al., “Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension,” American Journal of Physiology, vol. 262, no. 5, pp. L614–L620, 1992.
[6] S. T. Bonvallet, M. R. Zamora, K. Hasunuma et al., “BQ123, an ETA-receptor antagonist, attenuates hypoxic pulmonary hypertension in rats,” American Journal of Physiology, vol. 266, no. 4, pp. H1327–H1331, 1994.
[7] V. S. DiCarlo, S.-J. Chen, Q. C. M. Qing Cheng Meng et al., “ETA-receptor antagonist prevents and reverses chronic hypoxia-induced pulmonary hypertension in rat,” American Journal of Physiology, vol. 269, no. 5, pp. L690–L697, 1995.
[8] D. D. Ivy, T. A. Parker, J. W. Ziegler et al., “Prolonged endothelin A receptor blockade attenuates chronic pulmonary hypertension in the ovine fetus,” Journal of Clinical Investigation, vol. 99, no. 6, pp. 1179–1186, 1997.
[9] N. Davie, S. J. Haleen, P. D. Upton et al., “ETA and ETB receptors modulate the proliferation of human pulmonary artery smooth muscle cells,” American Journal of Respiratory and Critical Care Medicine, vol. 165, no. 3, pp. 398–405, 2002.
[10] S. Soma, H. Takahashi, M. Muramatsu, M. Oka, and Y. Fukuchi, “Localization and distribution of endothelin receptor subtypes in pulmonary vasculature of normal and hypoxia-exposed rats,” American Journal of Respiratory Cell and Molecular Biology, vol. 20, no. 4, pp. 620–630, 1999.
[11] B. Rondelet, F. Kerboull, S. Motte et al., “Bosentan for the prevention of overcirculation-induced experimental pulmonary arterial hypertension,” Circulation, vol. 107, no. 9, pp. 1329–1335, 2003.
[12] J. Lutz, M. Gorenflo, M. Habighorst, M. Vogel, P. E. Lange, and B. Hocher, “Endothelin-1- and endothelin-receptors in lung biopsies of patients with pulmonary hypertension due to congenital heart disease,” Clinical Chemistry and Laboratory Medicine, vol. 37, no. 4, pp. 423–428, 1999.
[13] J. Wong, V. M. Reddy, K. Hendricks-Munoz, J. R. Liddicoat, R. Gerrets, and J. R. Fineman, “Endothelin-1 vasoactive responses in lambs with pulmonary hypertension and increased pulmonary blood flow,” American Journal of Physiology, vol. 269, no. 6, pp. H1965–H1972, 1995.
[14] D. D. Ivy, J. W. Ziegler, M. F. Dubus, J. J. Fox, J. P. Kinsella, and S. H. Alman, “Chronic intrauterine pulmonary hypertension alters endothelin receptor activity in the ovine fetal lung,” Pediatric Research, vol. 39, no. 3, pp. 435–442, 1996.
S. Ameshima, H. Golpon, C. D. Cool et al., “Peroxisome pro-
lial cells,” Chest, vol. 114, no. 1, supplement, p. 65, 1998.

G. Hansmann and R. T. Zamanian, “PPARgamma activation: a potential treatment for pulmonary hypertension,” Science Translational Medicine, vol. 1, no. 12, pp. 12–14, 2009.

N. Wang, L. Verna, N.-G. Chen et al., “Constitutive activation of peroxisome proliferator-activated receptor-γ suppresses pro-
lial cells,” Journal of Biological Chemistry, vol. 277, no. 37, pp. 34176–34181, 2002.

J. Li and N. Wang, “Peroxisome proliferator-activated receptor-
y in vascular biology,” Cardiovascular and Hematological Dis-
ders, vol. 7, no. 2, pp. 109–117, 2007.

E. K. Kim, J.-H. Lee, Y.-M. Oh, Y.-S. Lee, and S.-D. Lee, “Rosig-
litazone attenuates hypoxia-induced pulmonary arterial hyper-
tension in rats,” Respiratory, vol. 15, no. 4, pp. 659–668, 2010.

G. Hansmann, V. A. de Jesus Perez, T.-P. Alastalo et al., “An antiproliferative BMP-2/PPARγ/apoE axis in human and murine SMCs and its role in pulmonary hypertension,” Journal of Clinical Investigation, vol. 118, no. 5, pp. 1846–1857, 2008.

G. Hansmann, R. A. Wagner, S. Schellong et al., “Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-γ activation,” Circulation, vol. 115, no. 10, pp. 1275–1284, 2007.

P. Delerive, F. Martin-Nizard, G. Chinetti et al., “Peroxisome proliferator-activated receptor activators inhibit thrombin-
uced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway,” Circulation Research, vol. 85, no. 5, pp. 394–402, 1999.

F. Martin-Nizard, C. Furman, P. Delerive et al., “Peroxisome proliferator-activated receptor activators inhibit oxidized low-
density lipoprotein-induced endothelin-1 secretion in endothelial cells,” Journal of Cardiovascular Pharmacology, vol. 40, no. 6, pp. 822–831, 2002.

T. Attinà, R. Camidge, D. E. Newby, and D. J. Webb, “Endothelin antagonism in pulmonary hypertension, heart failure, and beyond,” Heart, vol. 91, no. 6, pp. 825–831, 2005.

A. P. Davenport, “International Union of Pharmacology—29, Update on endothelin receptor nomenclature,” Pharmacological Reviews, vol. 54, no. 2, pp. 219–226, 2002.