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Recombinant human relaxin reduces hypoxic pulmonary hypertension in the rat

Carol A. Tozzi[a,1], George J. Poiani[a,2], Nansie A. McHugh[a,3], Michael P. Sharkarjian[a], Beverly H. Grove[b], Chrishan S. Samuel[c], Elaine N. Unemori[b], David J. Riley[a,*]

[a]*Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854-5635, USA*
[b]*Connetics Corporation, 3400 West Bayshore Road, Palo Alto, CA 94303, USA*
[c]*The Howard Florey Institute of Experimental Physiology and Medicine, The University of Melbourne, Royal Parade and Grattan Street, Parkville, Vic. 3010, Australia*

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Abstract

The fibroproliferative changes in pulmonary artery (PA) remodeling are partially prevented by antifibrotic agents. Relaxin (Rlx), a hormone involved in loosening collagen bundles in ligaments during parturition, has antifibrotic and vasodilator properties that may prevent pulmonary vascular remodeling. In the hypoxia model of pulmonary hypertension, two doses of recombinant human relaxin (rhRlx 24 [high] or 5 [low] $mg \times 10^{-2}/kg\ d^{-1}$) were administered subcutaneously continuously for 10 d to hypoxic (10% O_2) rats. At day 11, right ventricular pressure ($Pa \times 10^2$) was reduced by rhRlx in a dose-dependent manner (15 ± 1* control; 28 ± 1 hypoxia; 23 ± 1* low; 20 ± 1* high; $n = 10$–14/group, *$P < 0.05$ vs. hypoxia). High rhRlx ameliorated increased collagen accumulation (μg hydroxyproline/vessel) in main PAs (87 ± 6) vs. untreated hypoxia (102 ± 2) ($n = 5$/group, $P < 0.05$). Infusion of rhRlx had no effect on air-breathing rats, and acute administration did not alter blood pressure in hypoxic rats. Fibroblasts cultured from rat PAs spontaneously expressed collagen and fibronectin, and treatment with TGF-β increased secretion 26- and 25×10^{-1}-fold, respectively. Addition of rhRlx to transforming growth factor-β-stimulated fibroblasts inhibited collagen (37%) and fibronectin (38%) secretion vs. vehicle ($n = 4$ per group, both $P < 0.05$). We conclude that rhRlx inhibits the early fibroproliferative response in hypoxic pulmonary hypertension and the mechanism may be due in part to suppression of collagen synthesis.

Keywords: Hypertension; Pulmonary; Collagen; Relaxin; Vascular remodeling; Fibrogenesis

1. Introduction

Pulmonary hypertension occurs in a variety of lung diseases that result in severe, sustained hypoxema. In animal models of pulmonary hypertension produced by exposure to hypoxia, hypoxic vasoconstriction occurs and is followed by structural remodeling which contributes to further elevation of pulmonary artery (PA) pressure. Major structural changes include hypertrophy and hyperplasia of smooth muscle cells and fibroblasts and accumulation of extracelluar matrix (ECM) proteins [1]. In the rat hypoxia model of pulmonary hypertension, studies suggest that excessive accumulation of collagen impairs normal function of the PA and contributes to pulmonary hypertension. Administration of agents that block collagen deposition ameliorate the development of pulmonary hypertension [2–4], and the amount of excess collagen in newly remodeled PAs is highly correlated with PA pressure and vascular compliance [5]. An attractive therapeutic approach to the treatment of pulmonary hypertension is administration of agents that modulate or inhibit collagen synthesis. Several agents have been studied which are targeted to inhibit procollagen genes, transcription of

* Corresponding author. Tel.: +1 732 235 5172; fax +1 732 235 2809.
E-mail address: riley@umdnj.edu (D.J. Riley).
[1] Present address: Merck & Co., RY34-B364, P.O. Box 2000, 126 E, Lincoln Avenue, Rahway, NJ 07065, USA.
[2] Present address: 7805 Shady Grove Road, Mt Crawford, VA 22841, USA.
[3] Present address: Schering-Plough Research Institute, 2015 Galloping Hill Road, K15-1-1600, Kenilworth, NJ 07033-1300, USA.

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mRNAs, or post-translational enzymes involved in collagen synthesis [6]. Although some of these agents have been shown to successfully inhibit fibrosis in animal studies, their potential for use in humans is not known.

Relaxin (Rlx) is a peptide hormone that is thought to be involved in remodeling of interpubic ligaments and cervix prior to parturition in the rat, decreasing or loosening collagen bundles in the ligament [7]. Relaxin also has demonstrated ability to block over-production of ECM molecules, including collagen and fibronectin, in fibroblasts derived from skin [8], lungs [9], and kidneys [10]. In rodents, Rlx administration is antifibrotic in fibrosis models of the skin [11], lung [9,12], and kidney [13,14]. In addition, it has recently been demonstrated that Rlx knockout mice develop cardiac [15] and pulmonary [16] fibrosis, both of which are reversible by administration of rhRlx. Relaxin has also demonstrated activity as a vasodilator in the kidney [17,18] and other vascular beds [19–22] via a mechanism of action believed to involve nitric oxide [17,22]. Because nitric oxide has shown promise in the treatment of pulmonary hypertension [23,24], Rlx has the potential to ameliorate vasoconstriction in the chronic hypoxia model. In addition, recent studies demonstrated that chronically administered Rlx altered the passive mechanics of renal arteries, indicating that Rlx may modify structural elements of the vascular wall, e.g. extracellular matrix proteins [25].

For these reasons, Rlx may be beneficial in the treatment of pulmonary hypertension. In this study, we determined whether rhRlx administration could inhibit the deposition of collagen in PAs in the rat hypoxic model of pulmonary hypertension and whether it would reduce PA pressure and resultant changes in right ventricular weight. Adventitial fibroblasts have been implicated as one of the cell types involved in vascular remodeling of hypoxia-induced pulmonary hypertension [26]. Because of this, we also examined the ability of rhRlx to inhibit the profibrogenic effects of the peptide transforming growth factor-β (TGF-β) [27] on collagen and fibronectin synthesis in isolated rat pulmonary artery fibroblasts.

2. Materials and methods

2.1. Animals and reagents

Outbred 6-week-old male Sprague–Dawley rats, 200–220 g body weight, were obtained from Hilltop Laboratories, Scottdale, PA. Recombinant human relaxin (5.0 mg/ml in 20 mM sodium acetate, pH 5.0) and vehicle (20 mM sodium acetate, pH 5.0) were provided by Connetics Corporation (Palo Alto, CA). Osmotic minipumps were purchased from Alza Corporation (model 2002, Alza Corp., Palo Alto, CA). The protocols were approved by the Institutional Animal Care and Use Committee of UMDNJ-Robert Wood Johnson Medical School.

2.2. Groups and preparation of infusion pumps

Osmotic minipumps were filled to deliver rhRlx at $24\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ (high rhRlx), $5\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ rhRlx (low rhRlx), or vehicle alone Osmotic minipumps were inserted subcutaneously in rats under pentobarbital sodium (50 mg/kg intraperitoneal (i.p.)) anesthesia. The area of the dorsum of the neck was shaved, cleaned with alcohol, and a small incision made for minipump insertion. Incision was closed with surgical staples. After the animals were fully conscious, they were assigned to either hypoxic exposure (h) or room (a) groups, as described below. Three groups of rats were subjected to air: $24\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ rhRlx (H-a), $5\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ rhRlx (L-a), and vehicle (V-a). Three groups were subjected to hypoxia: $24\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ rhRlx (H-h), $5\ \text{mg}\times10^{-2}/\text{kg d}^{-1}$ rhRlx (L-h), and vehicle (V-h).

2.3. Hypoxic exposure and hemodynamic measurements

Rats were exposed to normobaric hypoxia (10% O_2, 90% N_2) as previously described [4]. Control groups were exposed to air in the same room. Both groups were maintained at constant temperature (18–24 °C), relative humidity (45–50%), and a 12 h light–dark cycle. Hypoxic animals were fed standard chow (Prolab RMH3000, PMI Feed Co., St Louis, MO) and water ad libitum. To match for final body weights, control rats were fed the same amount of food as that consumed by pair-matched hypoxic rats and were given water ad libitum. Exposures were carried out for 10 d. On day 11, rats were removed from the chambers, and mean right ventricular pressure (RVP) was measured in rats anaesthetized with an i.p. injection of pentobarbital sodium (50 mg/kg). Mean RVP was measured by the procedure of Widimský and co-workers [28] with modifications [4]. Briefly, a polyethylene catheter (PE-90; 86×10^{-2} mm internal diameter; 127×10^{-2} mm outer diameter) was inserted into the right external jugular vein and advanced to the right ventricle. Pressure was measured by a pressure transducer (model P23Pb; Statham Instruments, Hato Rey, PR) and the output was recorded on a strip chart recorder (model SP-2006, Statham Instruments). After a 5 min stabilization period with the animal breathing air, phasic right ventricular pressures were measured for 10 min. Mean RVP was computed as the pressure half-way between maximal and minimal pressures. Data were excluded if the catheter was not in the right ventricle at autopsy. Measurements were made 20 min after the animals were removed from hypoxia, a time sufficient to reverse hypoxic vasoconstriction in chronically hypoxic rats [29]. The portal vein was then cut, and blood samples were collected for analyses of hematocrit and circulating rhRlx, as described below. After sacrifice, the ratio of the weights of right ventricle to left ventricle plus septum (RV/[LV + S]) was measured as previously described [4].