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AT_2 receptor agonist LP2 restores respiratory function in a rat model of bleomycin-induced lung remodelling

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ABSTRACT

This study aimed to evaluate the prophylactic and therapeutic potential of angiotensin II type 2 receptor peptide agonist LP2 in bleomycin-induced airway and cardiac remodeling in rats. Male Wistar rats were intratracheally instillated with bleomycin. Animals of a prophylactic arm received LP2 from day 0 at intraperitoneal doses of 1, 3 or 10 μg/kg/d, whereas animals from a therapeutic arm received this LP2 treatment from day 7. On day 28 direct lung mechanics were determined and cardiac and lung tissues were collected and (histo)morphologically assessed. Prophylactic LP2 at 1 μg/kg/d with bleomycin, versus bleomycin alone, significantly improved the airway pressure responses at fixed inflation of 4 ml ($p < 0.05$) and 7 ml volume ($p < 0.05$), static compliance ($p < 0.01$), inspiratory capacity ($p < 0.05$), lung tolerance of increased volume ($p < 0.0001$), right to left ventricular hypertrophy ($p < 0.05$). Therapeutic regime showed a similar trend as the prophylactic arm but was less effective, mostly lacking significance. However, and importantly, therapeutic LP2 at 1 μg/kg/d significantly decreased mRNA expression of collagen 1A1 ($p < 0.01$), of Connective Tissue Growth Factor 1 ($p < 0.05$) and of Tissue MetalloPeptidase inhibitor 1 ($p < 0.05$). In conclusion, a very low dose of 1 μg/kg/d LP2 has capacity to counter bleomycin-induced impairment of lung functioning and consequent cardiac remodeling.

1. Introduction

The renin angiotensin system plays an important and often undervalued role in lung function and offers relevant opportunities for therapeutic intervention with lung diseases. AT_1R stimulation causes vasoconstriction and renal retention of electrolytes and water, and also promotes inflammation, proliferation and fibrosis [1]. While unbalanced stimulation of the ACE/AngII/AT_1R axis may induce lung injury, the protective arms of the renin angiotensin system counter the AT_1R-mediated effects by exerting opposite effects. The ACE/AngII/AT_2R axis, the ACE2/Ang-(1−9)/AT_2R axis and the ACE2/Ang-(1−7)/MasR axis exert effects that are generally opposite to those of AT_1R, as demonstrated by multiple studies. For instance, cyclic angiotensin-(1−7), a MasR agonist, has shown beneficial effects in animal models of neonatal lung injury [2] and acute respiratory distress syndrome [3,4]. Overexpression of AT_2R may contribute to repairing lung injury [5]. Angiotensin-(1−9) ameliorates pulmonary arterial hypertension, a disease which involves fibrosis in the lung, via stimulation of AT_2R [6]. Stimulation of the AT_2R by C21 protects against cigarette smoke-induced chronic obstructive pulmonary disease [7,8].

Abbreviations: ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme type 2; ACEi, ACE inhibitor; Agtr1a, angiotensin II type 1a receptor; Agtr1b, angiotensin II type 1b receptor; Agtr2, angiotensin II type 2 receptor; ARB, AT_1R blocker; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type-2 receptor; Bleo, bleomycin; BW, body weight; Collagen1A1, component of type I collagen, called the pro-alpha1(I) chain; C_{st}, Static compliance; CTGF1, Connective Tissue Growth Factor 1; CVF, Collagen Volume Fraction; BPM, Breaths Per Minute, respiratory rate; ET, Expiratory Time; i.p, Intraperitoneally; IC, Inspiratory Capacity; IPF, Idiopathic Pulmonary Fibrosis; IT, Inspiratory time; HPRT, Hypoxanthine PhosphoRibosylTransferase; LP2, lanthipeptide agonist of AT_2R; LV, Left Ventricle; MasR, Mas receptor; MrgD, Mas related G-protein coupled receptor D; MW, Minute Ventilation; PEF, Peak Expiratory Flow velocity; PIF, Peak Inspiratory Flow velocity; RV, Right Ventricle; RVH, Right Ventricle Hypertrophy; TGF-β1, Transforming Growth Factor β1; TIMP1, Tissue MetalloPeptidase inhibitor 1; TT, Total respiratory Time; TV, Tidal Volume.

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Imbalanced renin angiotensin system in COVID-19, can be restored by agonists of MasR and AT_2R [9,10]. Administration of AT_2R-agonist C21 can be beneficial in hypoxia-induced pulmonary hypertension in rats [11]. Fibrosis is a shared feature of many lung diseases. Idiopathic pulmonary fibrosis is a major unmet medical need. The bleomycin-induced rat model, used in this study, is a well-established model of lung fibrosis [12].

LP2 (Fig S1) is a lanthipeptide AT_2R agonist. Consistent with the generally enhanced target-selectivity of constrained peptides, and on the basis of a multitarget safety panel, lanthionine-constrained LP2 appears selective for AT_2R [13]. Consistent with this selectivity, LP2 showed excellent safety and pharmacokinetics in a clinical phase Ia trial [13]. LP2 previously showed antifibrotic [2] and anti-inflammatory effects [2] in a neonatal hyperoxia induced rat pup model of lung fibrosis. Here we studied in rat whether prophylactic and/or therapeutic LP2 could counter bleomycin-induced airway disease and/or cardiac remodelling.

2. Material and methods

2.1. Synthesis of LP2

LP2 is a lanthipeptide with the sequence dKDRV$[dAIHA]_s$ in which dK is a D-lysine, dA a D-alanine and $[dA—A]_s$ a D, L lanthionine composed of two alanines with a thioether bridge (Fig S1). LP2 was synthesized via base-assisted sulfur extrusion of a D,L disulfide bridged precursor peptide, dKDRVdCIHC in which dK is a D-lysine and in which dC is a D-cysteine [13].

2.2. Animals

All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Research Council's Guide for the Care and Use of Laboratory Animals. Seven- to eight-week-old male Wistar rats weighing 226 – 250 g (Charles River Laboratories) were allowed to acclimate for a minimum of one week before enrolment into the study. The gene encoding AT_2R, the target of the agonist LP2, lies on the X-chromosome [14]; subsequent studies with female rats will be needed. Animals were provided ad libitum access to standard chow (Harlan 8640) and water and housed under standard conditions.

2.3. Treatments

On day 0 all rats (prophylactic and therapeutic arm) were instilled intra-tracheally with vehicle (PBS) or 1.25 mg/kg bleomycin (Plato Biopharma) formulated in vehicle. Body weight was monitored daily. LP2 formulated in PBS was administered intraperitoneally at 0.5; 1.5 and 5 μg/kg every 12 h to achieve final doses of 1, 3 and 10 μg/kg/day. On day 0, animals from the prophylactic arm started to receive LP2, while all remaining rats were treated with vehicle. On day 7, animals allocated to the therapeutic arm started to receive LP2. Before, these animals underwent whole body plethysmography, which along with body weight data allowed to allocate the rats to functionally matched treatment groups (Table 1).

2.4. Pulmonary function

Non-invasively measured pulmonary function parameters, including respiratory rate, volumes of inhalation and exhalation, as well as the time required for a full breath, were assessed using whole body plethysmography and analysed with Ponemah software (DSI, St Paul, MN). Endpoint lung pressure-volume curves have been obtained on the final day of study, day 28. Briefly, rats have been anesthetized with 5 % isoflurane, and maintained on 2 % isoflurane in 100 % oxygen. Following tracheotomy, animals received pancuronium bromide 1.5 mg/kg i.p. to inhibit voluntary respiratory efforts and transferred to a FlexiVent ventilator (Scireq, Canada) for the direct assessment of pressure-volume relationships.

Table 1
Treatment scheme for prophylactic and therapeutic arms.

Controls	Treatment	Treatment schedule [day numbers]	Number of rats	Bleomycin dose [mg/kg]	LP2 dose [μg/kg/day, i.p.]
	Control	n/a	8	n/a	n/a
	Bleo	n/a	16	1.25	n/a
Prophylactic	Bleo/LP2	0–28	17	1.25	1
	Bleo/LP2	0–28	17	1.25	3
	Bleo/LP2	0–28	17	1.25	10
Therapeutic	Bleo/LP2	7–28	15	1.25	1
	Bleo/LP2	7–28	15	1.25	3
	Bleo/LP2	7–28	15	1.25	10

n/a not applicable.

2.5. Blood

For blood and morphological analyses, the right jugular vein and descending aorta were cannulated with polyethylene tubing, and following collection of terminal blood from the arterial cannula, the pulmonary vasculature was perfused with oxygenated 0.9 % NaCl to remove all blood while the animal's heart remained beating. Then, the heart and lungs were harvested, immersed in ice-cold 0.9 % NaCl, dissected, and subjected to subsequent analyses.

2.6. Histology

For histological lung analyses the left lung was inflated to 25 cm of pressure with 10 % Neutral Buffered Formalin, isolated and immersed in 10 % Neutral Buffered Formalin for 48 h, then transferred to 70 % ethanol prior to histological processing. The fixed left lungs were grossly dissected in apical (cephalad), mid- and basal (caudal) regions, embedded to paraffin blocks and sliced with a microtome into 5 μm sections. For pulmonary Collagen Volume Fraction (CVF) sections were stained with Masson's trichrome blue, using standard methods. Quantitative histological analyses were done using standard colour spectral segmentation analyses. Images (n = 10/region/lung) of Masson's trichrome blue-stained lung sections were obtained in a blinded manner using a Zeiss AxioImager.A2 at 200x. The low and high signals of the spectral segmentation mask were developed using vehicle control and vehicle-treated bleomycin groups, respectively; values from all treatment groups were obtained in a blinded manner. CVF was expressed as the average positive stain across sampled images. One CVF composite score was calculated for each individual animal i.e. (CVF depth 1 + CVF depth 2 + CVF depth 3) / 3. Group CVF scores were calculated from the individual CVF composite per animal and represented as the mean CVF composite score per group.

2.7. mRNA

For lung mRNA analysis, biopsies of the right ventricle and the entire pulmonary artery trunk were collected on wet ice and flash frozen in liquid nitrogen. The samples were homogenized with a Qiagen TissueLyzer II (Qiagen, Germantown MD) bead mill using standard homogenization buffer. Molecular profiling was performed with QuantiGene 2.0 Plex Assay Kit QP1011 (Thermo Fisher Scientific) on the Luminex platform according to the manufacturer's instructions. All gene products (α-SMA, Collagen 1a1, CTGF1, TGF-β1, TIMP-1, Agtr1a, Agtr1b, Agtr2, and angiotensinogen) were quantitated and normalized to HPRT expression as an internal housekeeping analyte.

2.8. Statistics

All statistical analyses were performed using GraphPad Prism software (GraphPad Software, Inc. San Diego, CA). Treatment groups were compared with One-way ANOVA followed by Dunnett's multiple comparisons test. Two factor analyses were computed with two-way ANOVA followed by Dunnett's multiple comparisons test. All data are expressed as mean ± SEM.

3. Results

3.1. Functional grouping of therapeutic arm

Body weight (BW) was measured in the different groups of the prophylactic (Fig. S2A) and therapeutic experiments (Fig. S2B). On day 7, animals from the therapeutic arm were equally grouped based on body weight (BW) and whole-body respiratory measures. At initiation of LP2 administration, BW of animals receiving LP2 + bleomycin was not different from the vehicle + bleomycin group but was significantly lower than that of the vehicle control group without bleomycin (Fig. S2AB). At initiation of LP2 administration, respiratory parameters (PIF, PEF, BPM, IT, ET, TT, TV, MW) did not differ from vehicle+-bleomycin control (data not shown).

3.2. Body weight

In study days 3–28 bleomycin instillation significantly reduced absolute BW in prophylactic and therapeutic arms as compared to the vehicle-treated animals (Fig. S2AB). At the study end on day 28, BW of bleomycin-treated rats in the prophylactic and therapeutic arms was significantly lower by approximately 13 % as compared to the vehicle-treated animals ($p < 0.001$). In the prophylactic arm, bleomycin-instilled animals receiving LP2 tended to gain more weight than vehicle-treated bleomycin rats over the same time period. However, except of a single timepoint (Fig. S2A, day 22, mean BW: Bleo 330 g vs. Bleo/1 μg LP2 361.3 g, $p < 0.05$), the differences were not significant. In the therapeutic arm, no significant differences between bleomycin-instilled animals receiving either LP2 or vehicle were observed (Fig. S2B).

Relatively to the baseline, all animals in the prophylactic and therapeutic arms gained BW (Δ BW) as assessed at the study end (Fig. S3A and S3B). Gain in body weight in vehicle treated animals was significantly higher than in bleomycin-treated animals (Δ BW: 87 g vs 36 g; $p < 0.05$). Prophylactic LP2 at 1 μg/kg/d was associated with a higher numerical gain in BW than bleomycin, but without reaching significance (Fig. S3A).

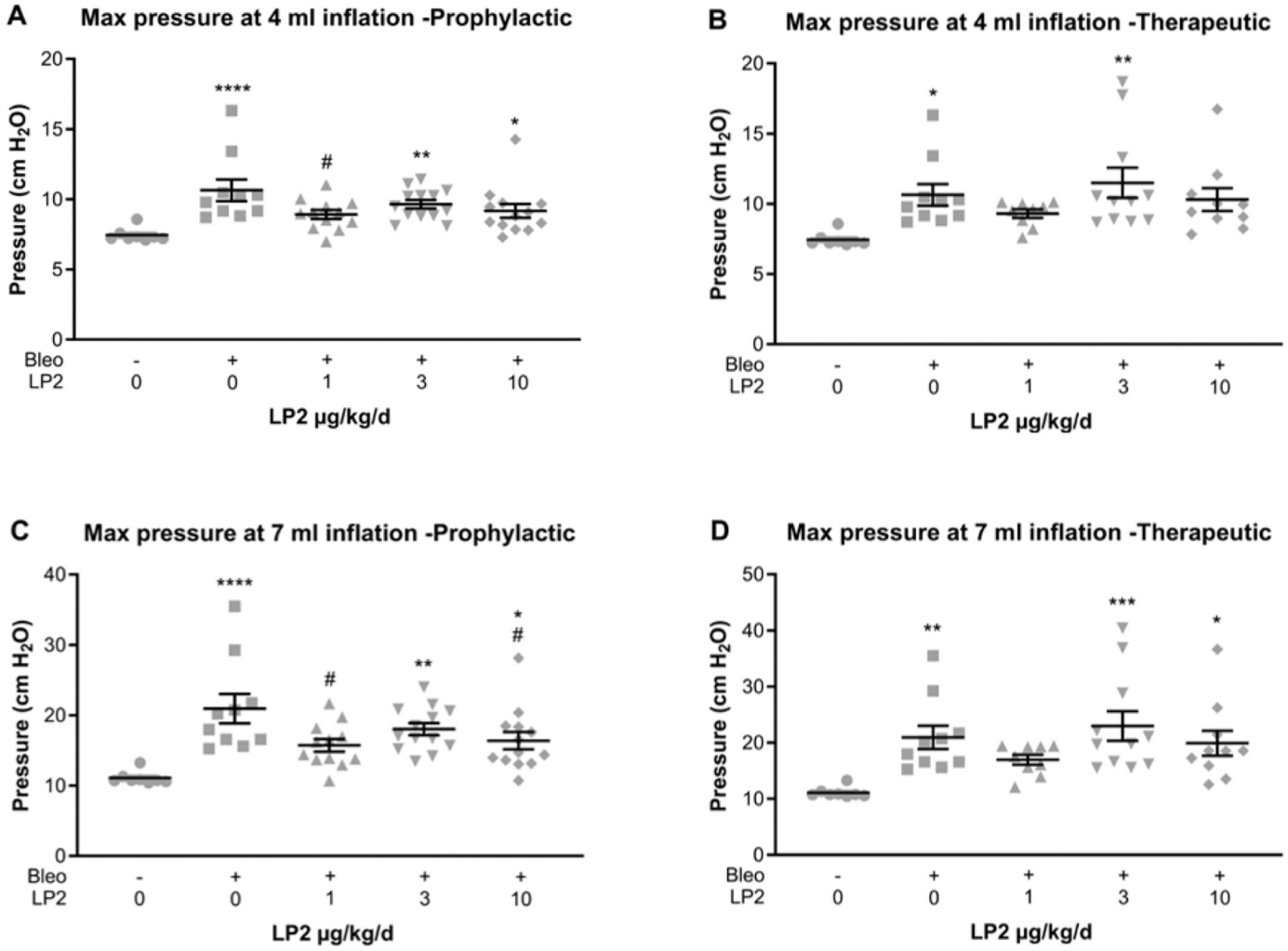

Fig. 1. ABCD. Endpoint direct lung mechanics -airway pressure to low and high fixed inflation. One-way ANOVA with Dunnett's multiple comparisons test. *p < 0.05 vs. Veh; **p < 0.01 vs. Veh; ***p < 0.001 vs. Veh; ****p < 0.0001 vs. Veh; # p < 0.05 vs. Bleo.

3.3. Lung weight

Bleomycin, compared to vehicle, caused a significant increase in lung weight as (Fig. S4A, S4B). LP2 did neither reduce the bleomycin-induced increase in lung weight (Fig S4A, S4B), nor when lung weight was normalized to tibia length (Fig. S4C, S4D).

3.4. Endpoint pulmonary mechanics

3.4.1. Prophylactic LP2 improves airway responses to inflation

In the prophylactic arm, the airway pressure responses to low level fixed inflation (set volume 4 ml; Fig. 1A) were significantly increased in bleomycin controls compared to vehicle. 1 μg LP2 significantly reduced this increase (mean pressure 8.92 vs. 10.64 cmH_2O; $p < 0.05$). In the therapeutic arm, no significant differences between bleomycin and LP2-treated animals were observed (Fig. 1B). These data suggest that in the prophylactic treatment 1 μg LP2 is effective and the optimal tested dose.

Similarly, in the prophylactic arm, the airway pressure responses to high level fixed inflation (set volume 7 ml; Fig. 1C) were significantly increased in vehicle treated bleomycin controls. Prophylactic 1 μg and 10 μg LP2 significantly reduced ($p < 0.05$) airway pressure responses compared to high level fixed inflation in bleomycin controls. Again, the LP2 treatment arm did not show significant differences compared to bleomycin alone (Fig. 1D). These data demonstrate efficacy of prophylactic LP2 with respect to airway pressure responses to high level fixed inflation.

3.4.2. Prophylactic LP2 improves static compliance

Static compliance (C_{st}) was calculated from pressure-volume curves and indicates the intrinsic elastic properties of the lung and chest wall. Compliance was significantly reduced in bleomycin groups compared to vehicle control. Prophylactic 1 μg LP2 significantly increased C_{st} ($p < 0.01$) (Fig. 2A). In the therapeutic arm, no significant differences between bleomycin controls and LP2-treated animals were observed (Fig. 2B).

3.4.3. Prophylactic LP2 increases inspiratory capacity

Inspiratory capacity (IC), the maximum volume of air that can be inhaled from tidal volume end-expiratory level, is impaired in bleomycin-induced lung injury [15]. In the prophylactic arm, inspiratory capacity was significantly reduced in bleomycin groups compared to vehicle control (Fig. 2C). Prophylactic 1 μg/kg/d LP2 significantly increased IC compared to bleomycin ($p < 0.05$). In the therapeutic arm, no significant LP2-mediated effect was measured (Fig. 2D). These data demonstrate that prophylactic LP2 may improve the inspiratory capacity by partly preventing or restoring bleomycin-induced lung injury.

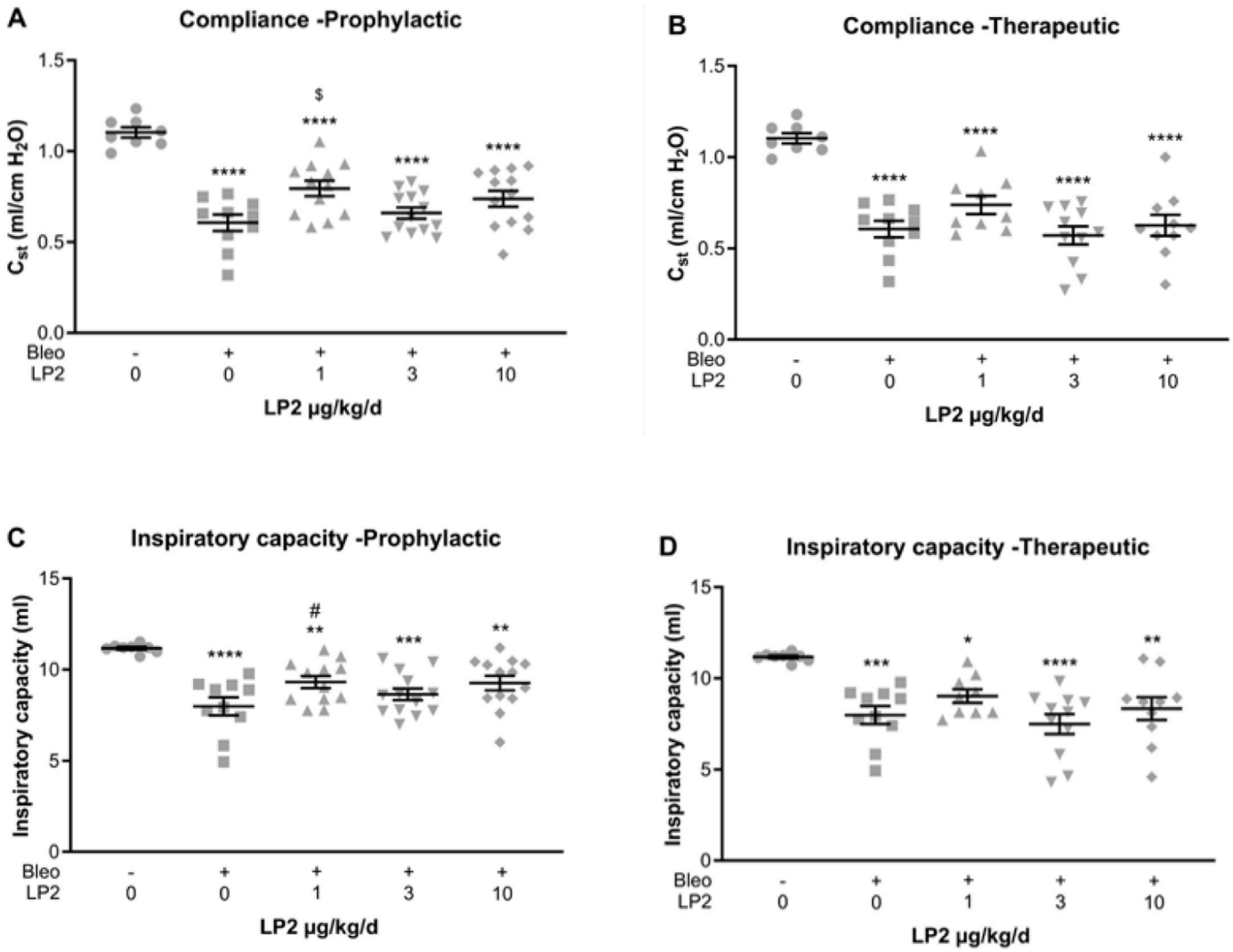

Fig. 2. ABCD. Endpoint direct lung mechanics -compliance and inspiratory capacity. One-way ANOVA with Dunnett's multiple comparisons test. *p < 0.05 vs. Veh; **p < 0.01 vs. Veh; ***p < 0.001 vs. Veh; ****p < 0.0001 vs. Veh; $p < 0.01 vs. Bleo; # p < 0.05 vs. Bleo.

3.4.4. Prophylactic LP2 improves lung tolerance of increased volume

Bleomycin injury caused a significantly increased pressure at higher volume compared to controls without bleomycin (Fig. 3AB). Prophylactic LP2 partly but significantly ameliorated the bleomycin-induced lung damage by restoring the volume/pressure relationship closer to that of the control without bleomycin. Prophylactic LP2, at all three doses, was significantly effective at volumes > 6.11 ml (Fig. 3A). In contrast, therapeutic LP2 was only significantly effective at a volume of 8.32 ml and only at a dose of LP2 of 1 μg/kg/d (Fig. 3B). These data demonstrate that all three doses of prophylactic LP2 are effective in partly restoring the volume pressure relationship.

3.5. Lung fibrosis

To assess lung fibrosis, the pulmonary collagen volume fraction (CVF) sections were stained with Masson's trichrome blue followed by

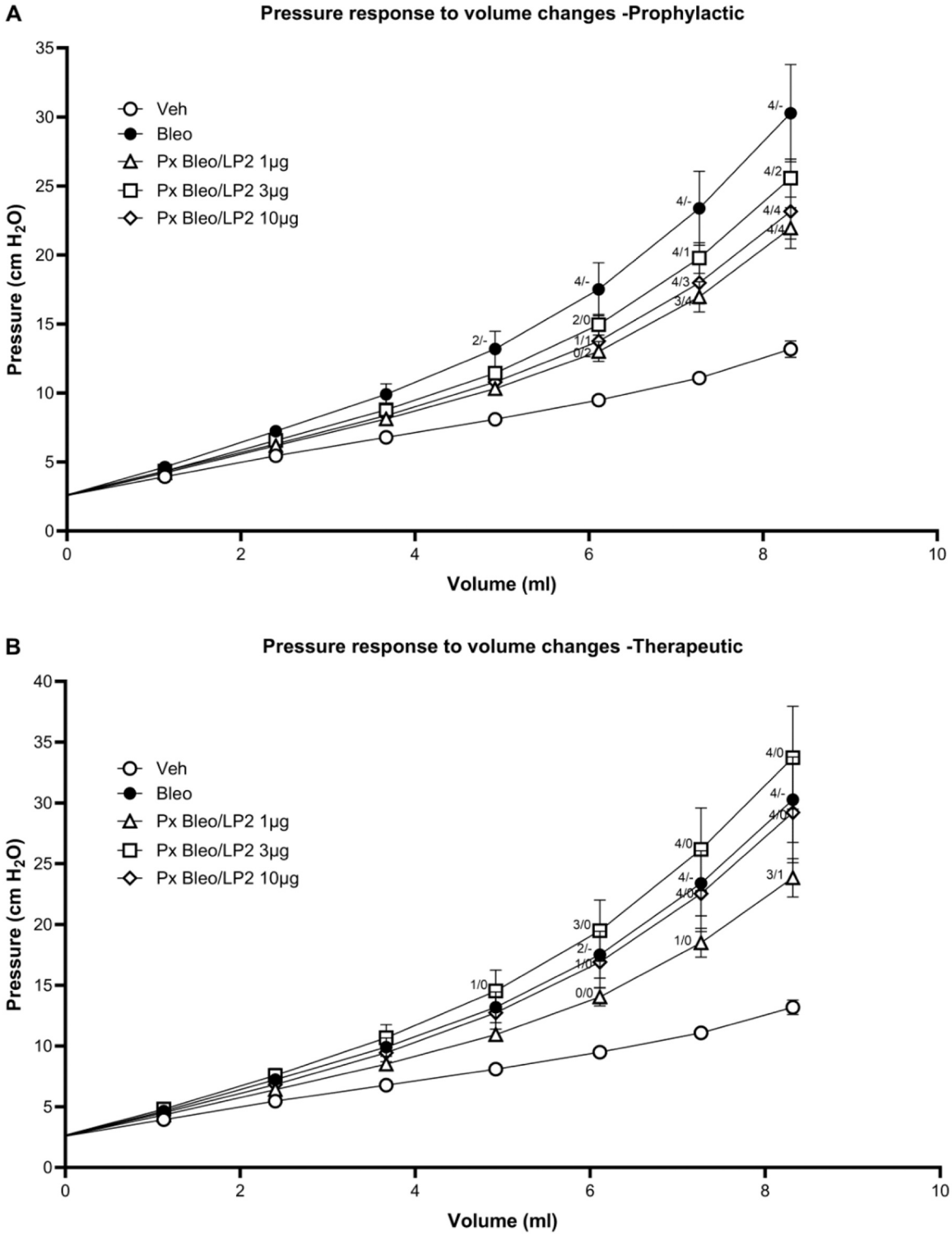

Fig. 3. AB. Endpoint direct lung mechanics -pressure response to step volume changes. Two-way ANOVA with Dunnett's multiple comparisons test. X/- vs. Veh; -/Y vs. Bleo; 1 $p < 0.05$; 2 $p < 0.01$; 3 $p < 0.001$; 4 $p < 0.0001$; - n.a.

quantitative histological analyses using colour spectral segmentation. Based on the above-mentioned results, the 1 μg/kg/d LP2 treatment group was selected for analysing lung fibrosis and lung mRNA expression (Fig. S5ABCD).

In the apical section (Fig. S5A), medial section (Fig. S5B), basal section (Fig. S5C), and composite CVF (Fig. S5D), the CVF area was significantly elevated in bleomycin-treated animals. Neither prophylactic LP2 not therapeutic LP2 did significantly reduce the bleomycin-induced increase in CVF.

3.6. mRNA expression of key molecules in the lung

Molecular profiling of fibrosis- and renin-angiotensin-related gene expression was performed on Luminex platform with QuantiGene 2.0 Plex Assay and results were normalized using the HPRT housekeeping gene. Collagen 1A1 mRNA expression (Fig. 4A) was significantly elevated only in the bleomycin group ($p < 0.01$) as compared to the vehicle-treated group. Compared to animals only receiving bleomycin, a significant reduction of collagen 1A1 mRNA was observed in the therapeutic group of 1 μg/kg LP2 ($p < 0.01$).

No significant differences between treatment groups and vehicle were present for CTGF1 mRNA expression (Fig. 4B). Compared to animals only receiving bleomycin, a significant reduction of CTGF1 mRNA was observed in the therapeutic group of 1 μg/kg LP2 ($p < 0.05$).

For α-SMA mRNA expression no significant effects of treatments were observed (Fig. 4C). For TIMP1 mRNA expression no significant differences between treatment groups and vehicle were present (Fig. 4D). Compared to the bleomycin group, therapeutic 1 μg/kg/d LP2 significantly ($p < 0.05$) reduced the expression of TIMP1 (Fig. 4D). Therapeutic 1 μg/kg/d LP2 seemed to increase expression of TGF-β1 compared to the bleomycin group (Fig. 4E). Expression of mRNA of $AT_{1a}R$, $AT_{1b}R$, AT_2R and angiotensinogen was neither significantly altered when comparing the vehicle group with the bleomycin-treated group, nor when comparing the bleomycin-treated group with the bleomycin-and-LP2-treated group, except for therapeutic LP2 at 1 μg/kg/d (not shown), which reduced mRNA expression of $AT_{1b}R$ compared to the bleomycin-treated group.

3.7. Cardiac morphometry

In animal models of bleomycin-induced lung injury, as a consequence of reduced lung functioning, changes of cardiac morphology can occur, often characterized by right ventricular hypertrophy [16]. In the current study, no differences in total heart weight between treatment groups and controls were observed neither for the prophylactic (Fig. 5A) nor for the therapeutic arms (Fig. 5B).

For the prophylactic arm, bleomycin caused a significant increase in the right to left ventricular weight ratio, a sensitive parameter also known as Fulton's index, an indicator of right ventricular hypertrophy [16] (Fig. 5C). LP2 at 1 μg/kg/d and at 3 μg/kg/d partly restored the bleomycin-induced changes by a significant reduction of the Fulton index versus bleomycin ($p < 0.05$ for both doses of LP2). For the therapeutic arm, LP2 did not significantly reduce the Fulton index (Fig. 5D). Hence prophylactic LP2 partly restores the (indirect) damage to the heart. It cannot yet be concluded whether this effect of LP2 is the result of LP2 action on the heart or whether it is an indirect effect via improving lung function or whether LP2 acts in this case both on the heart and the lung.

The left ventricular weight in the prophylactic arm was not affected; neither by bleomycin nor by combined bleomycin and LP2 treatment (Fig. S6A). In the therapeutic arm (Fig. S6B), LV weight was significantly reduced only in the 3 μg/kg/d LP2 group ($p < 0.05$) as compared to the vehicle treatment.

For both the prophylactic and therapeutic treatment arms, bleomycin caused a significant increase in the absolute right ventricular weight (Fig. S6C, S6D) and the RV:LV tibia length-normalized right ventricular weight (Fig. S6E, S6F) as compared to the vehicle-treatment. In both treatment arms LP2 at 1 μg/kg/d seems to reduce this bleomycin-induced increase, however, not significantly.

In the prophylactic study bleomycin caused a significant increase in atria weight compared to the vehicle (Fig. S6G). LP2 did not significantly reduce this bleomycin induced increase in atrial weight. In the therapeutic study atria weight was significantly increased in the 10 μg/kg/d LP2 group compared to vehicle control (Fig. S6H). Taken together, bleomycin affects cardiac morphology and some of the parameters are partly restored by prophylactic 1 μg/kg/d LP2, either by direct action on the heart, or indirectly via improving lung function.

4. Discussion

Lung fibrosis and in particular idiopathic pulmonary fibrosis (IPF) is a serious, but rare disease [17]. IPF is a progressive and terminal lung disease, with median survival of about 3–5 years after diagnosis, without any known cure. IPF is characterized by high deposition of extracellular matrix proteins by lung fibroblasts and myofibroblasts, resulting in reduced gas exchange and impaired pulmonary function. While symptoms of lung fibrosis can be treated, the reversal of fibrotic tissue to functional lung tissue remains an unmet medical need.

The lung contains high levels of ACE, an enzyme that converts Ang I to the active octapeptide Ang II. Increased ACE concentrations have been reported in the bronchoalveolar fluid in lung diseases [4]. Angiotensinogen is one of the most overexpressed genes in patients with pulmonary fibrosis. This cascade contributes to increased Ang II production during lung injury and fibrosis. Ang II stimulates the expression of (TGF)-β and connective tissue growth factor (CTGF), two potent pro-fibrotic factors that drive lung fibroblast/myofibroblast proliferation and ECM protein expression [18]. Detrimental effects in pulmonary fibrosis, including proliferative and pro-fibrotic actions on fibroblasts, may follow unbalanced stimulation of AT_1R by Ang II.

On the other hand, the renin angiotensin system also offers distinct therapeutic approaches for pulmonary fibrosis [19]. Well-established methods to limit the detrimental effects of AT_1R are the use of AT_1R blockers (ARBs) or the use of ACE inhibitors (ACEi). These methods are widely tested in models of pulmonary fibrosis [20]. Alternatively, instead of inhibiting AT_1R, selective stimulation of AT_2R / MasR / MrgD leads to therapeutic effects opposite to the detrimental effects of AT_1R.

Here we studied the efficacy of prophylactic and therapeutic treatment with the AT_2R-agonist LP2 in a bleomycin model of lung fibrosis in rat. With respect to most functional lung parameters prophylactic LP2 exerted significant effects and appeared more effective than therapeutic LP2. In contrast, with respect to expression of collagen 1A1, therapeutic LP2 caused a significant reduction whereas prophylactic LP2 did not. Furthermore, therapeutic LP2 lead to highly significant reductions of expression of Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP1) and Connective Tissue Growth Factor 1 (CTGF1).

Consistent with the generally enhanced target-selectivity of constrained peptides, lanthionine constrained peptide LP2 is highly selective for AT_2R also at high dose as evidenced by an in vitro multitarget safety panel, in which 10 μM of LP2 exclusively showed interaction with AT_2R only [13]. At the highest tested doses the efficacy of LP2 is lower, limiting the upper side of the prophylactic / therapeutic window in this indication. The lowest tested dose of 1 μg/kg/d appears to have the highest efficacy, which might leave open the positive scenario of higher efficacy at an even lower dose.

Few studies have explored the therapeutic potential of AT_2R agonism in pulmonary fibrosis: the AT_2R agonist C21 in a monocrotaline rat model of pulmonary hypertension reversed pulmonary fibrosis and prevented right ventricular fibros [21], and attenuated the progression of lung fibrosis and pulmonary hypertension in a bleomycin rat model of pulmonary fibrosis [22]; the AT_2R agonist LP2 reduced pulmonary inflammation and prevented right ventricle hypertrophy in hyperoxia-mediated acute lung injury in rat pups [2]. None of these

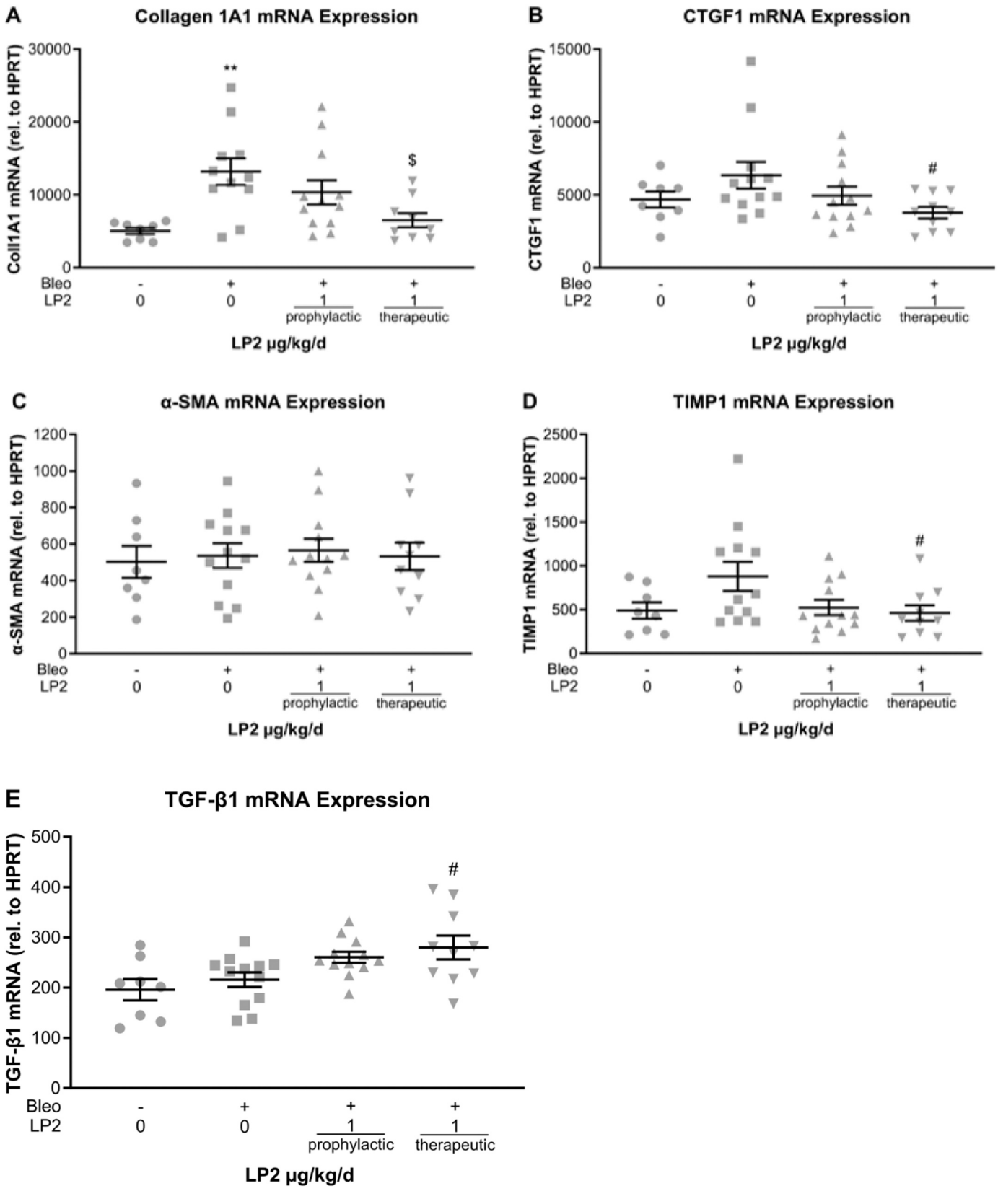

Fig. 4. ABCDE. Lung mRNA expression of profibrotic and tissue remodelling markers. One-way ANOVA with Dunnett's multiple comparisons test. **$p < 0.01$ vs. Veh; # $p < 0.05$ vs. Bleo; $ $p < 0.01$ vs. Bleo.

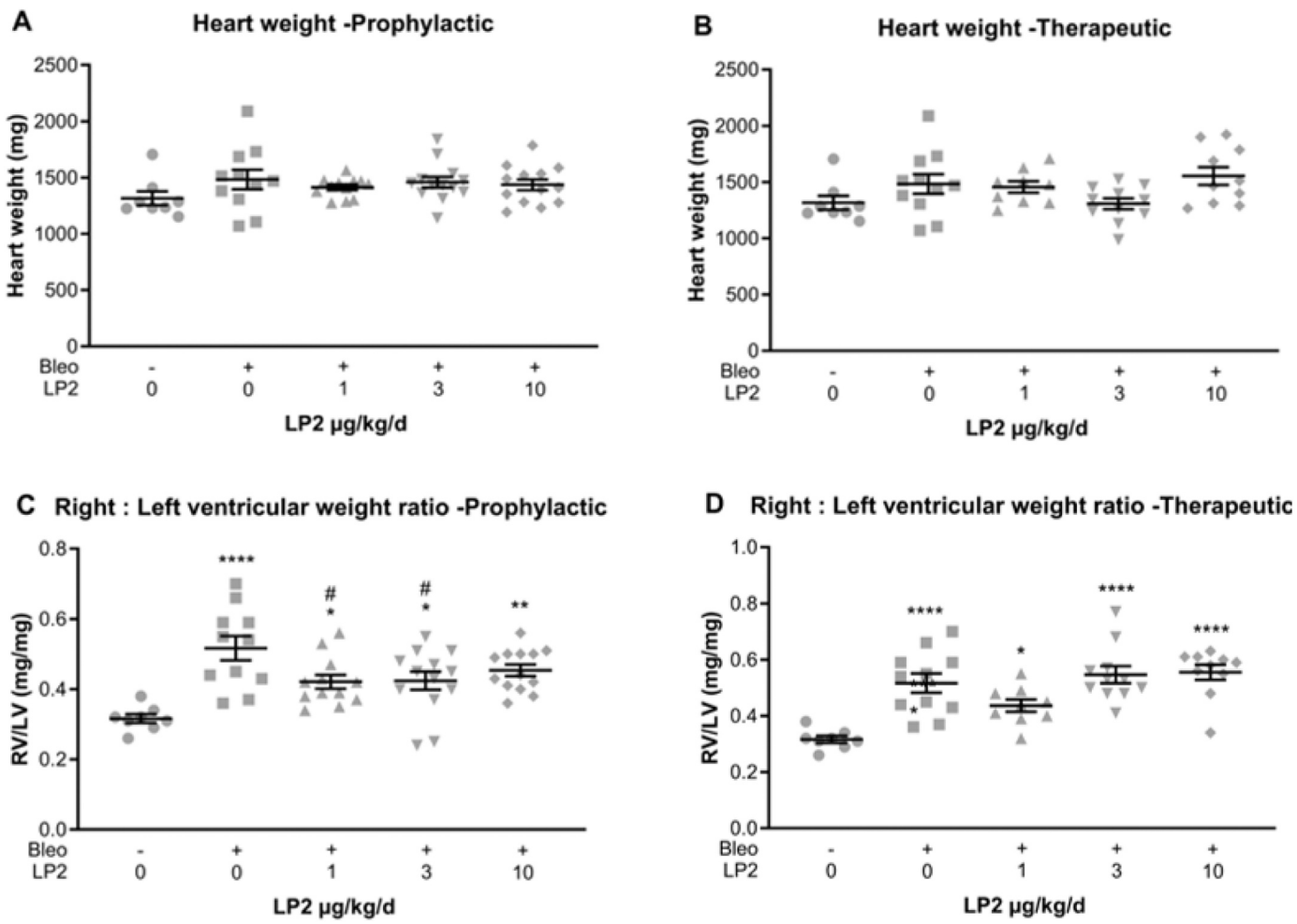

Fig. 5. ABCD. Cardiac weight and right to left ventricular weight ratio. One-way ANOVA with Dunnett's multiple comparisons test. *p < 0.05 vs. Veh; **p < 0.01 vs. Veh; ***p < 0.001 vs. Veh; ****p < 0.0001 vs. Veh; # p < 0.05 vs. Bleo.

studies has investigated the impact of AT_2R agonism on lung mechanics. For the treatment of pulmonary fibrosis, it is evident that there is a need for drug combinations with different therapeutic modalities that target several pathways involved in fibroproliferation [23].

Here, we assessed the impact of the AT_2R agonist LP2 on the endpoint lung mechanics under prophylactic and therapeutic treatment regimes. In the fibrotic phase after lung injury, fibrotic changes progress in the alveolar interstitium and the lung tissues become hardened [24]. As anticipated, bleomycin injury resulted in impaired lung physiology, as demonstrated by decreased inspiratory capacity and lung compliance. Compared to the bleomycin group, LP2 at 1 µg/kg/d significantly reduced airway pressure to low and high fixed inflation and improved compliance and inspiratory capacity in the prophylactic arm of the study. These results suggest that LP2 preserves the ability of the lungs to expand and reduces lung stiffness. A similar trend was observed in the therapeutic arm; however, no statistical significance was achieved. Measuring the elastic recoil of the lung with the quasi-static pressure-volume (PV) curve by inflating and deflating the lung in a stepwise fashion confirmed that the highest efficacy of LP2 was achieved at a low dose of 1 µg/kg/day. No significant differences in lung weight were observed between the treatment groups.

In addition to lung parameters, the effect of LP2 treatment on cardiac right ventricular hypertrophy (RVH) was assessed. Bleomycin intervention resulted in increased RVH as assessed by the right to left ventricular weight ratio, which was significantly reduced by LP2 at either 1 or 3 µg/kg/d in the prophylactic arm. Delayed LP2 treatment (therapeutic arm) resulted in a non-significant reduction of the RVH. Similarly, preventive and therapeutic (starting 3 days after bleomycin) treatment with C21 reduced RVH in BLM-instilled rats [22]. Thus, both studies support the beneficial role of pharmacological AT_2R stimulation in cardiopulmonary disease.

5. Conclusion

In conclusion, prophylactic LP2 may contribute to reducing airway remodelling especially with respect to lung function. By its low dose, safety and distinct mode of action, and potential for synergism, LP2 may qualify as a valuable add-on to standard therapies for lung disease.

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CRediT authorship contribution statement

All authors have contributed to discussion and evaluation of the obtained data and the writing of the manuscript. CR performed statistical analyses. DCV and GNM performed most of the writing of the manuscript. All authors have agreed on the final version of the manuscript.

Data Availability

Data will be made available on request.

Acknowledgment

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Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.peptides.2023.171106.

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