

Telmisartan Protect Broiler Chickens from Pulmonary Arterial Remodeling Induced by Low Ambient Temperature

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Abstract

Objective: Renin-angiotensin system is associated with many vascular diseases. This study aims to explore the protective effect of telmisartan, Angiotensin type 1 receptor blocker, on pulmonary arterial remodeling induced by low ambient temperature in broiler chickens.

Methods: Ninety chickens were randomly divided into 3 groups (n=30), including control group, low temperature group and telmisartan group. Chickens in low temperature group and telmisartan group were exposed to low ambient temperature from 14 days of age until 45 days of age; chickens in telmisartan group were gavaged with telmisartan 5 mg/kg once daily for thirty days. The pulmonary arterial systolic pressure was measured with right catheterization method. Wet lung weight index and right ventricular hypertrophy index were evaluated. Small pulmonary artery wall thickness and elastic fibers was determined respectively by H-E staining and Weigert staining. Proliferating Cell Nuclear Antigen (PCNA)-positive cells were determined by Immunohistochemical staining.

Results: It showed that pulmonary arterial systolic pressure, wet lung weight index and right ventricular hypertrophy index, small pulmonary artery wall thickness, elastic fibers and PCNA expression increased significantly in Low temperature group compared with control group and telmisartan group.

Conclusion: Telmisartan can protect broiler chickens from pulmonary arterial remodeling induced by low ambient temperature

Keywords: Renin-angiotensin system; Angiotensin type 1 receptor blocker; Telmisartan; Pulmonary hypertension; Right ventricular hypertrophy; Vascular remodeling; Medications

Introduction

Pulmonary Arterial Hypertension (PAH) is a debilitating disorder characterized by progressive narrowing of the pulmonary arterioles, which leads to increased resistance to the flow of blood from right ventricle to the lungs and eventually results in right ventricular failure and death. The disease is clinically defined as sustained elevation of the pulmonary artery pressures of >25 mmHg at rest or 30 mmHg during exercise, in absence of any underlying cause [1]. The pathology of pulmonary hypertension includes endothelial, smooth-muscle, and/or adventitial abnormalities, which result in obliterative remodeling of the pulmonary circulation. It is characterized by vasoconstriction, occlusion of the lumen in medium-sized and small pulmonary arteries due to excessive cellular proliferation in the vascular wall, and *in situ* thrombosis, with loss of microvessels and capillaries [1,2].

Pulmonary hypertension syndrome, also known as ascites, is a common disease occurred in broiler chickens under conditions such as high altitude or low ambient temperatures [3,4]. However, its mechanism is not clear. Studies show that dysregulated Renin-Angiotensin System (RAS) contributes to increased pulmonary vascular remodeling, and local RAS-upregulation was associated with increased pulmonary artery smooth muscle cell proliferation via enhanced angiotensin type 1 receptor (AT1-R) signaling in patients with idiopathic pulmonary arterial hypertension [5,6]. Angiotensin (Ang) II, the major biologically active component of RAS, acts through AT1 receptor and AT2 receptor (AT2-R). AT1-R is responsible for mediating many of the well-known stimulatory pathological actions of

Ang II including secretion of aldosterone, vasoconstriction, and renal sodium reabsorption. Telmisartan is the inhibitor of AT1-R. This study is designed to explore the effect of telmisartan on pulmonary arterial remodeling induced by low ambient temperature in broiler chickens.

Materials and Methods

Animals

Broilers chickens were purchased from Luoyang Chicken Alliance at 13 days of age. All animals were free to feed and water. Ninety broiler chickens were randomly divided into 3 groups (n=30): Control group, Low temperature group and Telmisartan group. Chickens in Control group were raised in normal temperature; Chickens in Low temperature group and Telmisartan group were exposed to low ambient temperature from 14 days of age until 45 days of age; at the same time, chickens in Telmisartan group were gavaged with telmisartan (Jiangsu nations biochemical medicine co., LTD; Approved by the enterprise: H20050715) 5 mg/kg by gavage once daily for thirty days. At 45 days of age, chickens were killed and samples were collected. The present

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study was conducted in accordance with the principles outlined in the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (<http://grants1.nih.gov/grants/olaw/>) and was approved by the local animal ethics committee at Henan University of Science and Technology.

Measurement of pulmonary arterial pressure

Pulmonary Arterial Systolic Blood Pressure (PASP) was measured with Right Catheterization Method⁶ using PCLAB-UE biomedical signal acquisition and processing system (Beijing Star Technology Development prestige limited liability company, Zhongguancun, Beijing, China). Chickens were anesthetized with pentobarbital (40 mg kg⁻¹). The catheter was inserted into right internal jugular vein through right atrium, right ventricle to pulmonary artery. The location of the catheter was judged by changes of waveform showed on the computer screen.

Evaluation of wet lung weight index

At 45 days of age, the Body Weight (BW) and Wet Lung Weight (wW) of chickens was measured. The wet Lung Weight Index (LI) was calculated using the formula: $LI = wW / BW$.

Evaluation of right ventricular hypertrophy index

Chickens were killed at 45 days of age, right and Left Ventricle Wall (RV and LV), ventricular Septum(S) was weighed and Right Ventricular Hypertrophy Index (RVHI) was calculated using the formula: $RVHI = RV / (LV S)$.

Haematoxylin-eosin (HE) staining

Lung tissue was collected and incubated in 10% Formaldehyde solution for one week, then embedded in paraffin wax; tissue was sliced into sections (4 μm) and HE staining was performed. The thickness of pulmonary arterial wall was measured by using image-pro plus software (Media Cybernetics, Silver Spring, MD, USA).

Weigert staining

Lung tissue was collected and incubated in 10% Formaldehyde solution for one week, then embedded in paraffin wax; tissue was sliced into sections (4 μm) and Weigert staining were performed. The integrated optical density of elastic fibers in pulmonary arterial wall was measured by using image-pro plus software (Media Cybernetics, Silver Spring, MD, USA).

Immunohistostaining

Lung tissue Proliferating Cell Nuclear Antigen (PCNA)-positive cells was identified by Immunohistostaining SABC method to determine the proliferative activity. Lung tissue was incubated in 4% paraformaldehyde for 24 hrs and embedded in paraffin wax. Sections (4 μm) were rinsed and rehydrated in phosphate buffered saline (PBS) for 5 min. Immunohischemical SABC detection kit (Wuhan Boster Bio-Engineering Co., Ltd, Wunan, China) was used to identify PCNA. Briefly, endogenous peroxidase was inhibited by treatment with 3% H₂O₂ in PBS for 10 min. Then, blocking solution with 5% BSA bovine serum albumin was applied to the sections for 15 min at room temperature to avoid non-specific binding of the biotinylated antibody. Antigen was repaired in boiling folic acid salt buffer (pH=6). Sections were incubated 37°C for 1 hrs and overnight with primary mouse anti-PCNA antibody (Beijing Biosynthesis Biotechnology Co., Ltd, Beijing, China). Labeling was identified by application of a rabbit anti-mouse IgG/HRP secondary antibody (Beijing Biosynthesis Biotechnology Co.,

Ltd, Beijing, China) 37°C for 30 min. Peroxidase activity was visualized using a DAB kit (Wuhan Boster Biological Engineering Co., Ltd, Wuhan, China). The reaction was stopped by rinsing in PBS. Finally, the slides were dehydrated, mounted in aqueous-based mounting medium and examined by light microscopy. The PCNA-positive cells of 20 fields of lung tissue at high magnification were counted and averaged.

Statistical analysis

Values are expressed as means ± SEM. One-way ANOVA and Tukey's post hoc test were used for all analyses; P<0.05 was considered significant. All analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Pulmonary arterial pressure

The Pulmonary Blood Systolic Pressure (PASP) was measured at 45 days of age. The pulmonary arterial pressure in low temperature group was higher than control group and telmisartan group ($p < 0.01$). There is no significant difference between telmisartan group and control group ($p > 0.05$) (Figure 1).

The Renin-Angiotensin System (RAS) is a major regulator of cardiovascular function. The dysregulated RAS plays a key role in the development of cardiovascular diseases [7,8]. Angiotensin II (Ang II), a core effector of RAS, is a multifunctional peptide with pleiotropic actions, modulating vasomotor tone, cell growth, senescence, apoptosis, migration and Extracellular Matrix (ECM) deposition [9,10]. The physiological effects of Ang II are mediated by Ang II receptor subtype 1 (AT1R). Elevated angiotensin II causes endothelial cells injuries and platelets deposition and oxidative stress. These initial events lead to expression of cytokines, chemokines secretion, and infiltration of inflammatory cells followed by myofibroblasts formation, leading to cardiac remodeling. In the present study, pulmonary hypertension was successfully induced by low ambient temperature in broiler chickens. It was found that AT1-R inhibitor, telmisartan, could decrease the pulmonary arterial systolic pressure significantly, which suggests that the activation of renin-angiotensin system might have involved in the pulmonary hypertension induced by low ambient temperature in broiler chicken.

Previous studies performed with AT1R antagonists have shown that AT1R blockage may improve endothelial function. The putative mechanism involved was the prevention of the inactivation of Nitric Oxide (NO) by superoxide anions. This hypothesis was supported by the finding that Nitric Oxide Synthase (NOS) inhibitor, L-N-monomethyl-arginine prevented the improvement in endothelial function seen with losartan and AT1R antagonists increased the antioxidative potential of the vessel wall by increasing the activity of endothelial superoxide dismutase [11]. In Baraunaa's study, it was found that telmisartan potentiated NO synthesis and blocked shear stress-induced AT1R activation. They showed evidences that telmisartan potentiate shear stress-induced NO production even in the absence of the ligand angiotensin II. This response requires both the inhibition of endothelial (eNOS) phosphorylation at its inhibitory residue Thr495 as well as the increase of eNOS phosphorylation Nitric Oxide Synthase at its excitatory residue Ser1177. In addition, the response is associated with inhibition of shear stress-induced ERK activation as well as elevated intracellular calcium transient [12]. Elevated intracellular Ca²⁺ triggers numerous signaling pathways including protein kinases such as the Calmodulin-Dependent Kinases (CaMKs) and the Extracellular

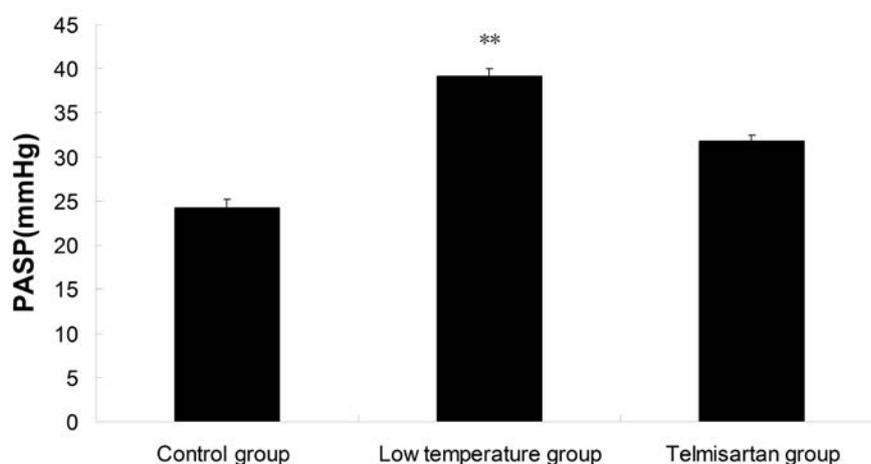


Figure 1: Telmisartan protects broiler chickens from pulmonary hypertension induced by low ambient temperature. Broiler chickens were randomly divided into 3 groups (n=8): Control group, Low temperature group and Telmisartan group. Chickens in Control group were raised in normal temperature; Chickens in Low temperature group and Telmisartan group were exposed to low temperature from 14 days of age; Chickens in Telmisartan group were gavaged with Telmisartan 5 mg/kg once daily until 45 days of age. Pulmonary Arterial Systolic Blood Pressure (PASP) was measured with Right Catheterization Method using PCLAB-UE biomedical signal acquisition and processing system. Data are presented as the mean \pm SD (n=8 in each group). **P<0.01 compared with Control group and Telmisartan group (one-way ANOVA).

	Control group	Low temperature group	Telmisartan group
LI	0.0051 \pm 0.0004	0.0064 \pm 0.0014*	0.0052 \pm 0.0002†
RVHI	0.2101 \pm 0.045	0.3788 \pm 0.1201**	0.2738 \pm 0.0579†

Right ventricular hypertrophy is depicted as the increase in RVHI compared with Control group and Telmisartan group. Data are presented as the mean \pm SEM (n=8 in each group). *P<0.01 compared with Control group; †P<0.05 compared with Control group; **P<0.05 compared with Low temperature group (one-way ANOVA).

Table 1: Effect of telmisartan on wet Lung Weight Index (LI) and Right Ventricular Hypertrophy Index (RVHI).

Signal-Regulated Kinases (ERKs). There is a “cross-talk” between these two protein kinase families [13]. Growing body of evidence has shown the function of Calcium/Calmodulin-Dependent Kinases (CaMKs) in cardiovascular pathophysiology. Santulli found that the loss of CaMK4 results in the development of hypertension, accompanied by its typical hallmarks: endothelial dysfunction, target-organ damage, and reduced survival rate. These suggest that CaMKs and ERKs might involve in the protective effect of telmisartan in pulmonary hypertension induced by low ambient temperature in broiler chickens [14].

Wet lung weight index and Right ventricular hypertrophy index

The wet lung weight index in low temperature group increased significantly when compared with control group ($p<0.01$), and it was reversed significant by telmisartan treatment ($p<0.01$) (Table 1). Right ventricular hypertrophy index in low temperature group increased significantly compared with control group ($p<0.01$), and it was reversed significant by telmisartan treatment ($p<0.01$) (Table 1).

The lung is characterized by double vascularization: the bronchial vasculature, deriving from thoracic aorta (intercostal and mammarian arteries), has a trophic role, while pulmonary system is part of air/blood barrier that plays respiratory function of the lung [15]. Broiler chickens typically hatch at a weight of 40 g and can grow to 4 kg which doubles almost 7 times within 8 weeks. The extremely rapid early growth performance of broilers imposes proportional challenges to their immature pulmonary and cardiovascular systems [16]. An inadequacy of vascular capacity for blood flow through the lung to provide the tissues with the oxygen needed for rapid growth is the primary cause of pulmonary hypertension-induced ascites. There are a variety of other

factors that can trigger the Ascites Syndrome (AS). These factors may cause increased blood flow because of a higher metabolic rate (cold, heat, certain nutrients, chemicals, etc.) [17,18]. The sequence of events resulting in the AS begins with a lack of oxygen for metabolism, which stimulates cardiac output causing increased vascular pressure in the lung and pulmonary arteries. This results in a higher pressure load on right ventricular muscle wall. The muscle cells respond by adding sarcomeres in parallel, causing thickening (hypertrophy) of the right ventricular wall [18].

In Wang’s study, blockade of the RAS by losartan partially prevented the augmented inotropic responses of the volume-overloaded failing hearts to isoproterenol, as well as upregulation of β -adrenoceptors (β -ARs) and redistribution of G Protein-Coupled Receptor Kinase (GRK) activity and isoform contents. The stimulation of the RAS has been reported to affect the sympathetic activity in different experimental models. Therefore, it is possible that the blockade of the RAS by AT1R blockers may depress the sympathetic system and produce the protective effects observed in this study [19].

Pulmonary arterial wall thickness and Elastic fibers

The pulmonary arterial wall in low temperature group was thicker than control group ($p<0.01$), and it can be reversed effectively by telmisartan treatment ($p<0.01$) (Figure 2A and 2B).

Pulmonary arterial elastic fibers were stained blue in Weigert staining. There is significant increase in low temperature group (Figure 3A and 3B).

The wall composition of arterioles has been described as consisting of three structurally distinct layers: intima, media and adventitia. The

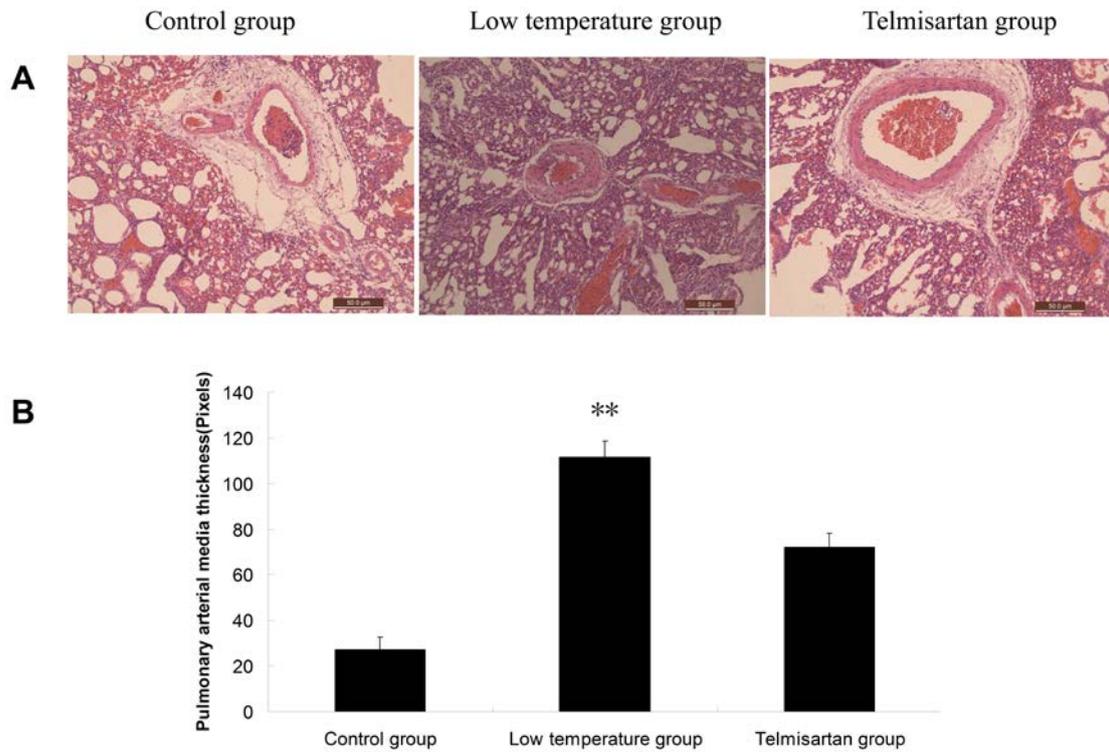


Figure 2: Effect of telmisartan on pulmonary arterial wall thickness. (A) The pulmonary arterial wall thickness was observed under light microscope. (B) Histograms represent the pulmonary arterial wall thickness. The pulmonary arterial wall thickness was measured by using image-pro plus software. Data are presented as the mean \pm SEM (n=8 in each group). ** P <0.01 compared with Control group and Telmisartan group(one-way ANOVA).

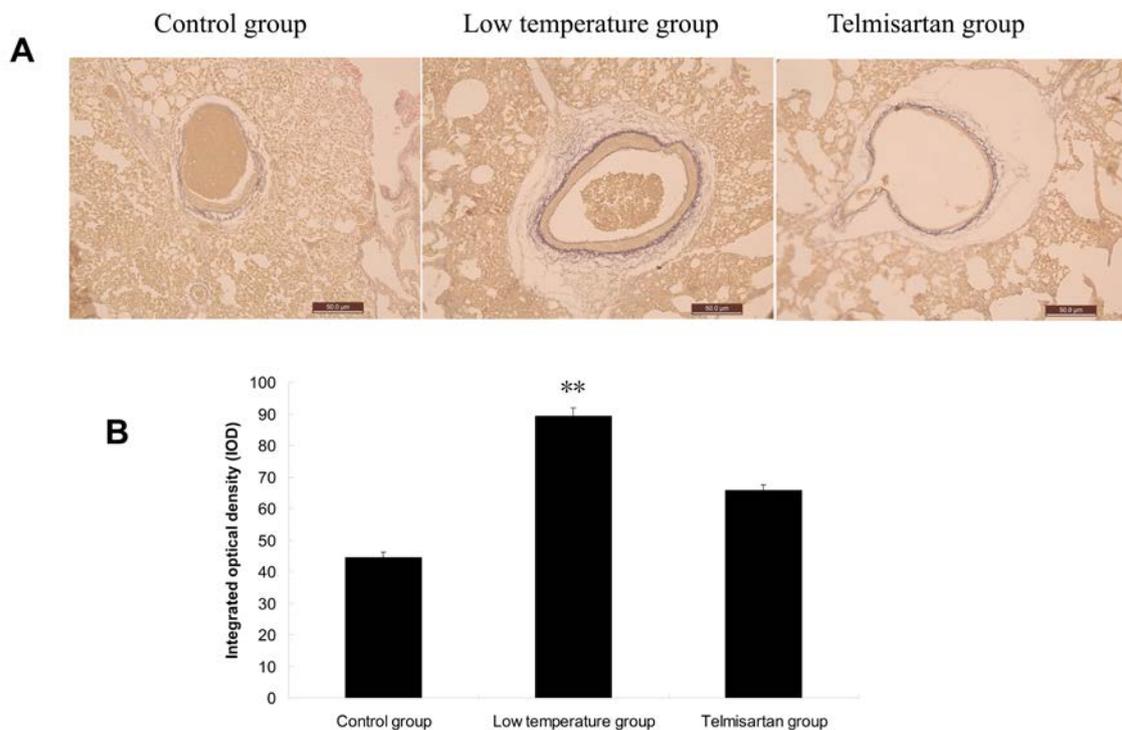


Figure 3: Effect of telmisartan on proliferation of pulmonary arterial elastic fibers. (A) Pulmonary arterial elastic fibers were stained blue in Weigert Staining. (B) Histograms represent integrated optical density (IOD) measured with image-pro plus software. Data are presented as the mean \pm SEM (n = 8 in each group). ** P <0.01 compared with Control group and Telmisartan group(one-way ANOVA).

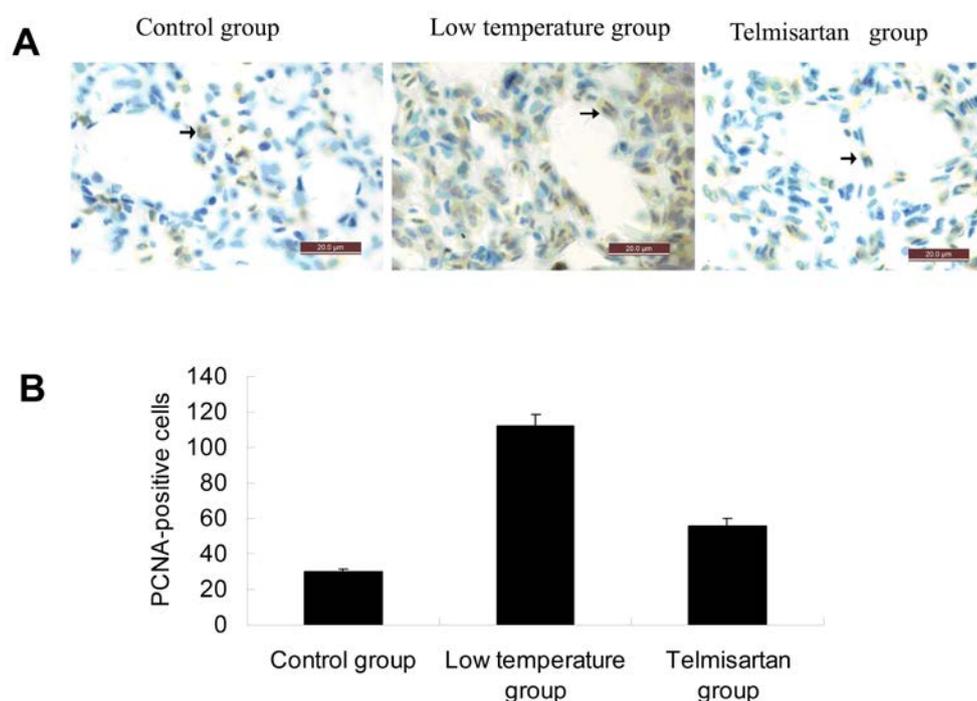


Figure 4: Effect of telmisartan on PCNA-positive cells in lung tissue. (A) Immunohistochemistry analysis: The PCNA-positive cells were observed under oil lens of microscope. (B) Histograms represent the number of PCNA-positive cells per visual field. The Ang II-positive cells of 20 fields of tubular area at high magnification were counted and averaged. Data are presented as the mean \pm SEM (n=8 in each group). * P <0.01 compared with Control group and Telmisartan group (one-way ANOVA).

medial layer in arterioles consists predominantly of vascular smooth muscle cells and an internal elastic lamina. The primary function of vascular smooth muscle cells within the media is to control vascular diameter via cell contraction and relaxation processes. Adventitial fibroblasts have been associated with the production of reactive oxygen species that in turn modulate the activity of smooth muscle cells in the vascular media and partake in the initiation of vascular remodeling [20-22]. Besides, angiogenesis might be another reason. Angiogenesis means the budding of new capillary branches from existing blood vessels. Increased angiogenesis also occurs in the lungs of patients with pulmonary hypertension, which is driven to a large extent by an exuberant proliferation of endothelial cells [15]. In this study, the pulmonary arterial wall was thickened in low temperature group, as well as elastic fibers, which suggest that low ambient temperature might have caused pulmonary arterial remodeling and endothelial cells proliferation, and it could be alleviated by telmisartan treatment. All these suggest that the RAS participates in the vascular remodeling induced by low ambient temperature in broiler chicken.

Lung tissue PCNA-positive cells

Lung tissue PCNA-positive cells in low temperature group increased significantly compared with control group, and it can be reversed effectively by telmisartan treatment (p <0.01) (Figure 4A and 4B).

PCNA is a cell cycle-dependent protein, the expression of which is usually considered as a reliable index of cell proliferation. In the present study, pulmonary arterial wall thickened in the low temperature group in comparison with control group, as well as the elastic fibers in arterial wall and PCNA-positive cells in lung tissue, and these could be prevented partially by telmisartan treatment. It

suggests that low ambient temperature might have activated lung tissue renin-angiotensin system and result in cell proliferation and arterial remodeling, which eventually contributed to pulmonary hypertension.

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