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Research on the improvement of oxidative stress in rats with high-altitude pulmonary hypertension through the participation of irbesartan in regulating intestinal flora

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Abstract. – OBJECTIVE: High-altitude pulmonary hypertension (HAPH) is one of the diseases with higher occurrence among people living in plateau areas. The possible mechanism of angiotensin II receptor 1 inhibitor irbesartan in improving HAPH was explored from the perspective of intestinal bacterial flora in this study.

MATERIALS AND METHODS: A HAPH rat model was established under simulated high-altitude hypobaric hypoxia. The levels of oxidative stress and vasoactive substances were detected after irbesartan intervention, and intestinal flora genomics analysis was performed.

RESULTS: High-altitude hypobaric hypoxia-induced the increase in pulmonary artery pressure and left ventricular systolic dysfunction in HAPH model rats, but its effects were alleviated by irbesartan. Changes in the levels of oxidative damage in intestinal tissues, such as the increase in superoxide dismutase and glutathione peroxidase in intestinal tissues and the decrease in malondialdehyde content, were also reversed by irbesartan. The serum levels of angiotensin II, endothelin 1, interleukin-6, and C-reactive protein increased substantially whereas the level of nitric oxide decreased in HAPH model rats. The levels of these vasoconstriction and inflammatory indicators were also reversed after irbesartan intervention. The distribution of intestinal florae in rats was changed by the simulated high-altitude hypoxia environment as manifested by the increased *Firmicutes*-to-*Bacteroidetes* ratio (F/B), the increased abundance of *Lactobacillaceae* and *Lachnospiraceae*, and the decreased abundance of *Prevotellaceae* and *Desulfovibrionaceae* at the family level. However, the changes in F/B ratio and the abundance of these florae were reversed by irbesartan.

CONCLUSIONS: Irbesartan can alleviate pulmonary artery pressure and left ventricular relaxation in HAPH model rats, reduce the oxidative damage caused by high-altitude hypoxia, and lower the release of vasoconstrictor factors and inflammatory mediators. These effects might be caused by the increased abundance of *Lactobacillaceae* and *Lachnospiraceae* and the decreased abundance of *Prevotellaceae* and *Desulfovibrionaceae* in the intestines.

Key Words:
High-altitude pulmonary hypertension, Irbesartan, Intestinal flora, Oxidative damage, Vasoactive substances.

Introduction

Approximately 140 million people live in plateau areas worldwide[1]. Plateaus have low air pressure and oxygen, which pose certain effects on the central nervous system, respiratory system, cardiovascular system, and hematopoietic systems of humans[2]. High-altitude pulmonary hypertension (HAPH) is a special disease that affects people living in high-altitude areas. The mechanism of this disease is believed to be the contraction and remodeling of pulmonary blood vessels, which ultimately lead to high-altitude cardiomyopathy[3]. Oxidative stress is involved in the occurrence of HAPH and exerts a certain effect on the remodeling of pulmonary blood vessels[4]. However, its mechanism is not yet clear. Current clinical drugs, such as carbonic anhydrase inhibitor (acetamidoamine), angiotensin-converting enzyme (ACE) inhibitor (enalapril), and dopaminergic antagonists and

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ventilation stimulants (medroxyprogesterone and almitrine), cannot achieve the ideal therapeutic effect and are accompanied by many adverse reactions, such as drug-induced liver and kidney damage[5,6]. Continuous stimulation under high-altitude hypobaric hypoxia is an important reason for the increased angiotensin II (AngII) synthesized by pulmonary vascular endothelial cells, the synthesis and release of endogenous vasodilator substances, and hypoxic pulmonary hypertension. AngII participates in oxidative damage, triggers inflammatory response, and increases the expression of other inflammatory response factors. AngII receptor type 1 inhibitor irbesartan has been widely used in essential hypertension and type 2 diabetes with hypertension but has not been studied in HAPH[7]. Therefore, irbesartan was chosen in this study.

Intestinal flora plays an important role in host health. Normal immune development and function will be impaired in the absence of intestinal flora[8]. Plateau environment is characterized by low atmospheric pressure and low oxygen partial pressure. Exposure to low-pressure hypoxia environment can lead to increased abdominal pain[9], infection risk and acute mountain sickness[10] and even gastrointestinal diseases[11]. Rodent research indicated that the dynamics of intestinal flora may contribute to the responses mentioned above, and exposure to hypobaric hypoxia can increase the risk of gastrointestinal damage, oxidative stress, and gastrointestinal permeability, which are accompanied by changes in the composition and activity of intestinal microbiota[12]. These observations indicate that intestinal microbiota may be affected by high-altitude hypobaric hypoxia and produce changes that adversely affect the body. However, more in-depth research on these relationships is needed to understand the impact of hypobaric hypoxia on the composition of intestinal microbial community, which is essential for regulating intestinal flora and improving intestinal health. However, changes in intestinal flora, oxidative stress, and vascular activity in HAPH have not been reported.

We hypothesized that irbesartan may regulate the oxidative/antioxidant balance in the body, restore vascular endothelial function, and reduce the level of inflammation in the body by adjusting the abundance of intestinal microbes. This study aimed to evaluate the effects of irbesartan on HAPH to provide a theoretical basis for the "new application of old medicine".

Materials and Methods

HAPH Animal Modeling Bbuilding and Irbesartan Intervention

Sixty 6-week-old male Sprague-Dawley rats (160-220 g) were raised in clean animal chambers with a temperature of 22 °C and a humidity of 50%. Photoperiod was 12 h, and water and food were given *ad libitum*. The rats in the control group (CG) were raised in a laboratory at an altitude of 800 m, whereas the rest of the rats were raised in a low-pressure chamber with a simulated altitude of 5000 m, a pressure of 54.1 kPa, and an oxygen partial pressure of 10.84 kPa for 45 days[13]. All rats were divided into six groups: control group (CG, n = 10), HAPH model group (MG, n = 10), nifedipine positive control group (NE, n = 10), irbesartan low-dose group (IB.L, n = 10), irbesartan medium-dose group (IB.M, n = 10), and irbesartan high-dose group (IB.H, n = 10). Nifedipine (YunPeng, Shanxi, China) or irbesartan (Sanofi, Hangzhou, China) was administered for 15 days after the rats were kept in hypoxic chambers for 30 days as follows: normal saline for the MG group; 2.7 mg/kg·day nifedipine for the NE group; and 6.75, 13.5, and 27 mg/kg·day irbesartan for the IB.L, IB.M, and IB.H groups, respectively (Figure 1).

Echocardiography Eexamination of Rat Heart

The rats were anesthetized by intraperitoneal injection of 0.75 mL/100 g solution of atropine (0.5 mg/mL), ketamine (50 mg/mL), and diazepam (5 mg/mL). Then, the rats were placed on an animal fixed plate in supine position, and their chest hair was removed. Color Doppler ultrasound apparatus (Philips, HDII XE, The Netherlands) with a 7.5 MHz probe (S12-4) was used for inspection. The four-chamber slices near the sternum and apex of the rats were routinely explored. Ventricular motion parameters were measured on the long-axis view of the left ventricle next to the sternum in rats by M-mode ultrasound.

Determination of Pulmonary Artery Pressure

According to the methods of Bogaard et al[14], the skin of the rats was cut down along the midline of the neck, and the subcutaneous and muscular tissues were obtusely separated to expose the trachea. An inverted T-shaped incision was made at the exposed part of the trachea, and the intubation tube was fixed and connected to the ventilator through

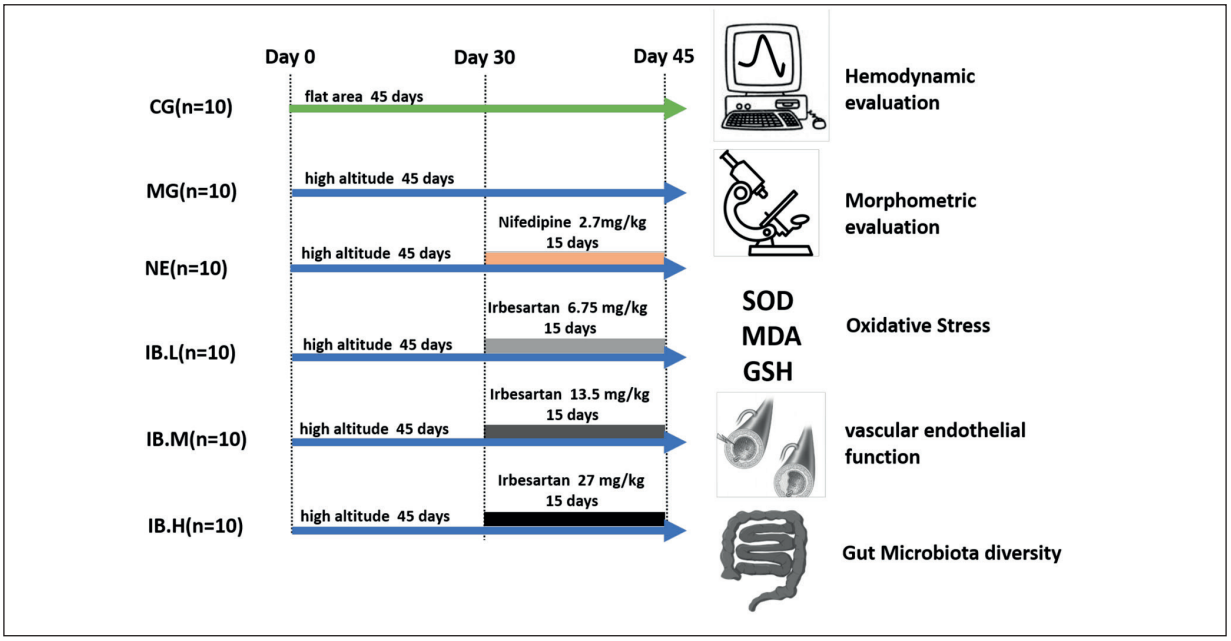

Figure 1. Schematic representation of the experimental protocols.

a Y-tube. Ventilator frequency was set to 60 times/min, tidal volume was 6 mL/kg, and respiratory ratio was 3:2. The thoracic cavity was cut along the midline of the rat's sternum to fully expose the lungs and heart. A heparinized saline needle was placed in the upper right corner of the right ventricle to measure pulmonary artery pressure and then connected to a pressure sensor in the other end to convert the pressure change into biological signal for the recording of experimental data.

Heart Hematoxylin and Eosin Staining

The left ventricle was cut longitudinally, and the pulmonary artery was cut transversely. Both parts were immediately cleaned with saline, fixed with 4% formaldehyde for 24 h, then washed with phosphate-buffered saline (PBS), and embedded in paraffin. The tissues were sliced into 4 μm-thick sections and stained with hematoxylin and eosin. The morphologies of the myocardium were observed through an upright microscope (Nikon, Tokyo, Japan).

Determination of Superoxide Dismutase (SOD), Malondialdehyde (MDA), and Glutathione Peroxidase (GSH-Px) Levels in Intestinal Tissues

About 1 g of rat intestinal tissue was weighed to make 10% tissue homogenate with 9 mL of frozen normal saline. The homogenate was centrifuged at 3000 r/min for 10 min, and the supernatant was saved after centrifugation. The total protein content of the tissue was determined through bicinchoninic acid assay (BCA). MDA level and SOD and GSH-Px activities (Nanjing Jiancheng, Jiangsu, China) were determined according to the methods provided in the instructions of the corresponding kits.

Determination of Rat Serum AngII, Interleukin-6 (IL-6), C-Reactive Protein (CRP), Endothelin 1 (ET-1), and Nitric Oxide (NO)

Pulmonary artery pressure was measured, and then blood from the abdominal aorta was obtained, placed at room temperature for 30 min, and centrifuged at 3000 r/min for 20 min in a low-temperature centrifuge. The supernatant was saved. AngII (USBiological, Swampscott MA, USA), ET-1 and NO (Nanjing Jiancheng, Jiangsu, China), IL-6 (eBioscience, San Diego, CA, USA), and CRP (Abcam, Cambridge, MA, USA) contents in rat serum were determined by enzyme-linked immunosorbent assay (ELISA) according to the corresponding kits' instructions.

Acquisition of Intestinal Contents and Analysis of Flora Composition

We analyzed the diversity of intestinal flora in the ileocecal contents of rats in the CG, MG,

and IB.H groups. The rats were sacrificed, and their ileocecal samples were obtained in sterile environment. About 1 g of ileocecal content was placed in a sterilized centrifuge tube, frozen quickly in liquid nitrogen, and then transferred to an −80°C refrigerator for storage. We extracted fecal genomic DNA using E.Z.N.A. Stool DNA Kit (Omega Bio-tek, GA, USA). The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGGAGGCAGCA-3′) and 806R (5′-ACTCCTACGGGAGGCAGCAG-3′) through polymerase chain reaction (PCR). PCR was carried out in a 20-μL system, which comprised 10 ng template DNA, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase (TransGen, Beijing, China), 2 μL of 2.5 μM dNTPs, and 4 μL of 5×FastPfu buffer. The PCR conditions were: 95 °C for 3 min; then 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s for 27 cycles; and a 10-min extension at 72°C. The product was purified with AxyPrep DNA gel extraction kit (Axygen Bioscience, San Diego, CA, USA) and quantified by Quantifluor-ST (Promega, Madison, WI, USA). The purified amplicons were sequenced on the Illumina MiSeq platform of Major Biomedical Technology Co., Ltd. according to standard protocol[15].

Statistical Analysis

Pair-end and double-ended sequences were spliced by FLASH (v1.2.11), and reads that could not be assembled were rejected for the analysis of microbial diversity[16]. Operational taxonomic units (OTUs) were clustered by UPARSE (version 7.1, http://drive5.com/uparse/), and sequences with 97% similarity and chimeric sequences were identified and removed by UCHIME[17]. Each sequence was classified and annotated according to the classification method of the Silva 16S rRNA database (https://www.arb-silva.de/) with 70% confidence threshold. Mothur (https://www.mothur.org/wiki/Download_mothur) was used to perform dilution analysis, compare α diversity index through dilution data, implement principal component analysis (PCA) graphs by R programming language, and conduct partial least squares discriminant analysis and mapping by R language mixOmics package[18].

The data are expressed as mean ± standard deviation apart from intestinal community analysis. One-way ANOVA and Student's *t*-test (SPSS 23.0; IBM, Armonk, NY, USA) were conducted, in which $p<0.05$ was considered statistically significant.

Results

Irbesartan Can Reverse the Hypertension and Left Ventricular Systolic Dysfunction Caused by Hypoxic Environment

The echocardiography results in Figures 2A and 3 show that the left and right systolic and diastolic diameters of the left ventricle in the

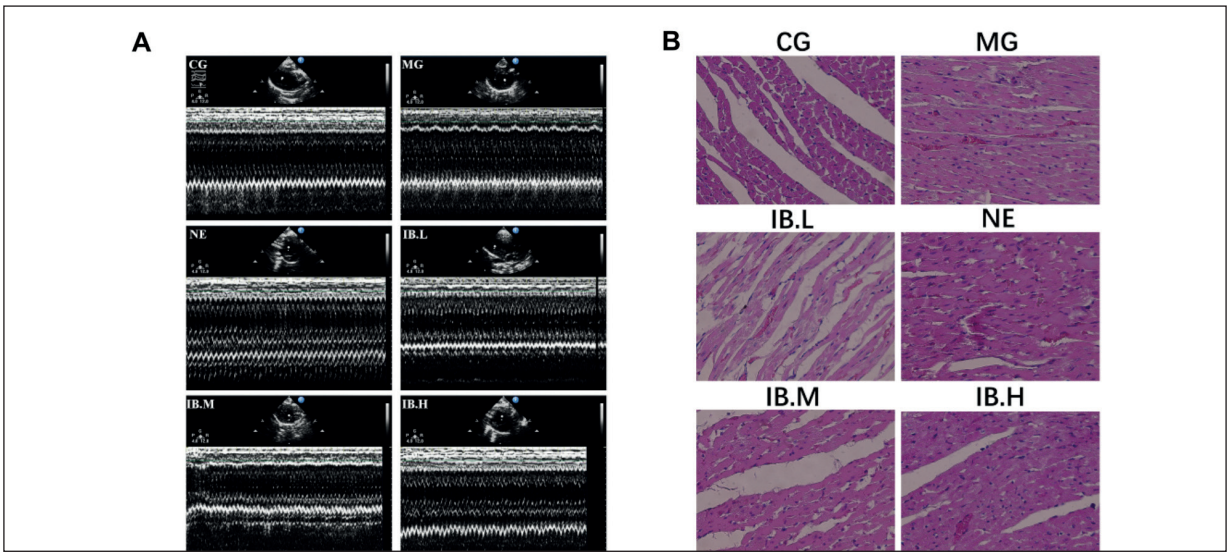

Figure 2. Irbesartan treatment attenuates ventricular systolic dysfunction and myocardial injury in HAPH rat. **A,** Echocardiography. **B,** Hematoxylin-eosin staining of myocardium (magnification: 100×).

MG group decreased in varying degrees, and the ventricular septum and the posterior wall of the left ventricle moved in the same direction compared with those in the CG group. This finding means that the systolic and diastolic functions of the left ventricle were affected by pulmonary hypertension. The right ventricle diameter increased, and the right atrium diameter decreased substantially after irbesartan intervention. The left ventricle diameter in the IB.M and IB.H groups increased during systole and diastole, whereas the left ventricle diameter in the IB.L group was smaller and not substantially different from the systole and diastole diameters in the CG group. This result shows that high- and median-dose irbesartan are effective in left ventricular diastolic dysfunction.

As shown in Figure 3A, the pulmonary artery pressure in the MG group increased significantly compared with that in the CG group (p<0.05). Compared with that in the MG group, the pulmonary artery pressures in the NE group and irbesartan-treated groups decreased significantly (p<0.05).

The results of hematoxylin and eosin staining are shown in Figure 2B. In the CG group, the structure of the heart tissue was normal and had no lesions; myocardial cells were stained clearly, and their nuclei were dense; and myocardial fibers were arranged neatly. In the MG group, myocardial interstitial blood vessels were significantly hyperemic, the horizontal stripes were unclear; part of the myocardium was swollen; and the cytoplasm underwent granular degeneration. The eosinophils of the myocardial fibers markedly degenerated, and occasional infiltration of inflammatory cells was observed. In the NE group, myocardial interstitial blood vessels were occasionally dilated and congested, transverse striation was unclear, myocardial cells were mild-

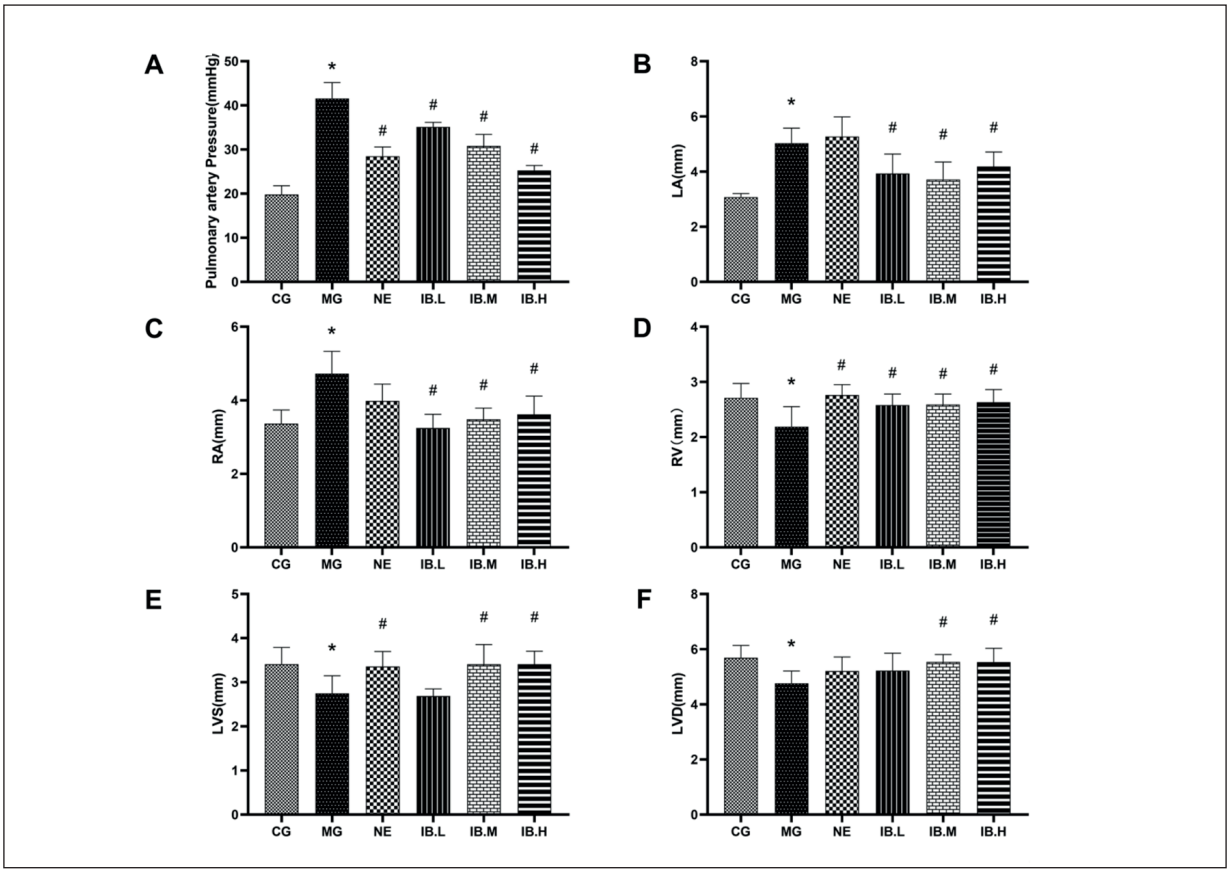

Figure 3. Irbesartan treatment attenuates elevated pulmonary artery pressure and echocardiographic indexes. **A,** Pulmonary artery pressure. **B,** Left atrium diameter. **C,** Right atrium diameter. **D,** Right ventricle diameter. **E,** Left ventricle systole diameter. **F,** Left ventricle diastole diameter. Data are expressed as means ± standard deviation (n = 10 per group). Differences in results among groups were calculated using ANOVA followed by Tukey's post-hoc test. *Means significant difference (p<0.05) compared with CG rat. #means significant difference (p<0.05) compared with MG model rat.

ly edematous, and a small number of myocardial cells underwent eosinophilic degeneration. In the IB.L group, significant diffuse hemorrhage was observed in some myocardial blood vessels, eosinophilic lesions were scattered, few interstitial inflammatory cells were found, and granular changes were occasionally seen. In the IB.M group, myocardial vascular bleeding was slight; a small amount of diseased eosinophilic cells and cell granular degeneration were seen, and myocardial fibers were arranged neatly. In the IB.H group, the myocardium was arranged regularly and had normal structure, a part of the myocardial interstitium was mildly hyperemic, and eosinophilic cells and inflammatory cell infiltration were seen.

Irbesartan Can Substantially Reduce the Oxidative Damage Caused by High-Altitude Hypoxia

Figure 4A shows that the MDA content in the MG group increased significantly compared with that in the CG group (p<0.05). The MDA contents of the IB.M and IB.H groups were significantly reduced compared with that in the MG group (p<0.05). The results suggest that MDA content can be substantially increased by high-altitude hypobaric hypoxia environment and effectively reduced by irbesartan. The IB.H group had the best performance in MDA reduction.

As shown in Figures 4B and 4C, the GSH-Px and SOD activities in the MG group were significantly reduced compared with those in the CG group (p<0.05). The GSH-Px and SOD activities in the NE group and irbesartan-treated groups increased significantly compared with those in the MG group (p<0.05). The results suggest that GSH-Px and SOD activities can be considerably reduced by the simulated plateau environment and effectively increased by nifedipine and irbesartan.

Irbesartan Can Improve the Vascular Endothelial Function Damage Caused by High-Altitude Hypobaric Hypoxia Environment

Figures 5A, 5B, 5D, and 5E show that AngII, ET-1, CRP, and IL-6 in the MG group significantly increased compared with those in the CG group (p<0.05). AngII, CRP, and IL-6 contents in the irbesartan-treated groups were significantly reduced compared with those in the MG group (p<0.05). Differences in ET-1 contents between the high- and medium-dose irbesartan groups and the MG group were statistically significant (p<0.05). The results suggest that AngII, ET-1, CRP, and IL-6 contents can be substantially increased by high-altitude hypobaric hypoxia environment and reversed by irbesartan. The IB.H group showed the best performance in improving vascular endothelial damage.

As shown in Figure 5C, the NO content in the MG group was significantly reduced (p<0.05) compared with that in the CG group, whereas the NO content in each dose group of irbesartan was significantly increased compared with that in the MG group (p<0.05). The results suggest that NO content can be significantly reduced by high-altitude hypobaric hypoxia and effectively increased by irbesartan. However, NO content cannot be remarkably increased by the positive control drug nifedipine.

Community Richness and Diversity Analyses

A total of 669,367 high-quality sequences were obtained from 30 intestinal flora samples

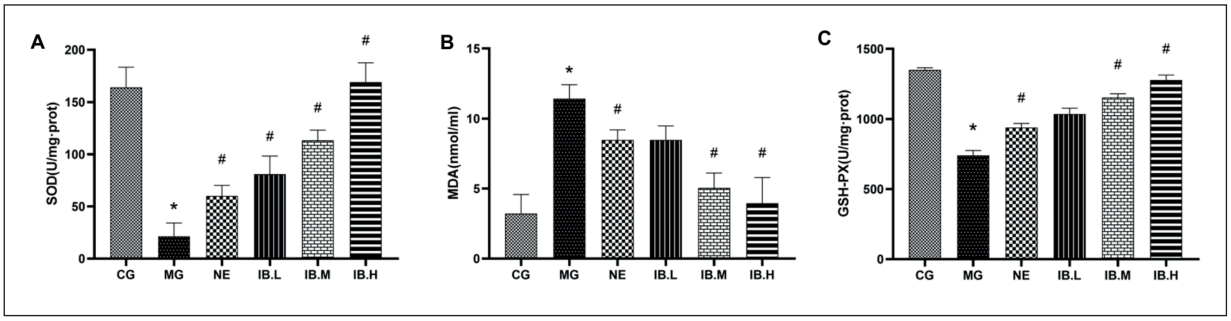

Figure 4. Irbesartan treatment attenuates oxidative stress in the intestinal tissue of HAPH rat. **A,** MDA level. **B,** GSH-Px level. **C,** SOD activity. Data are expressed as means ± standard deviation (n = 10 per group). Differences among groups were calculated using ANOVA followed by Tukey's post-hoc test. *Means significant difference (p<0.05) compared with CG rat. #means significant difference (p<0.05) compared with MG model rat.

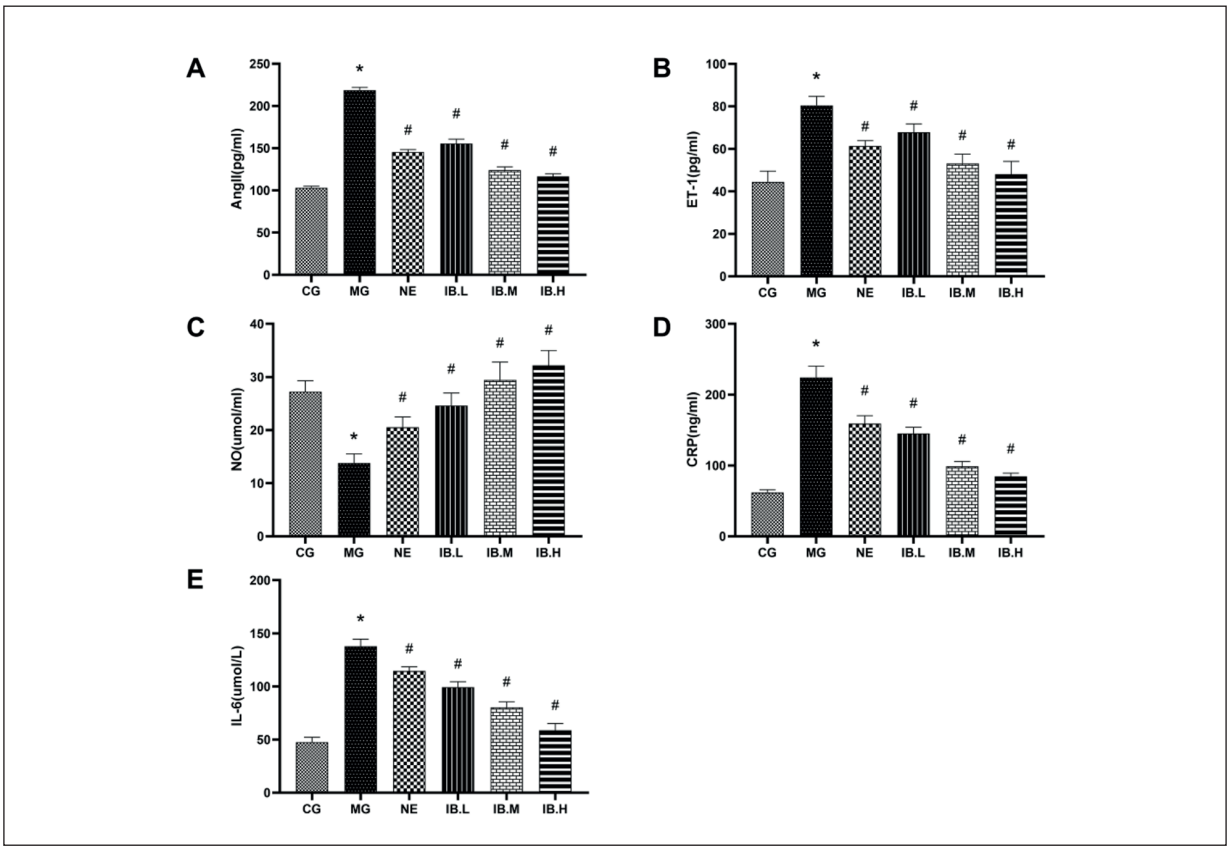

Figure 5. Effect of irbesartan on the release of vasoactive mediators and inflammation markers in rat plasma. **A,** AngII concentrations. **B,** ET-1 concentrations. **C,** NO concentrations. **D,** CRP concentrations. **E,** IL-6 concentrations. Data are expressed as means ± standard deviation (n = 10 per group). Differences among groups were calculated using ANOVA followed by Tukey's post-hoc test. *Means significant difference (p<0.05) compared with CG rat. #means significant difference (p<0.05) compared with MG model rat.

with an average of 37,187 sequences per sample. The high-quality sequences were divided into 1108 OTUs with 97% similarity. The quality of sequencing was evaluated using the dilution curve method based on the α diversity index, and result showed that the sequencing data was sufficient to cover all species in the microbial community.

The observed number of colonies (Sob), Chao index, Shannon index, Simpson index, and other ecological parameters were adopted to evaluate the composition of bacterial community. Sob and Chao index estimate the number of OTUs contained in the sample (Figures 6A and 6B). The Shannon and Simpson indexes reflect the diversity of the community, particularly the uniformity of the distribution of individuals in the group (Figures 6C and 6D). The Sob and Chao indexes between the groups were not substantially different; thus, the number of observed bacterial species and their community richness was not affected by hypoxia and irbesartan. However, the Shannon index increased whereas the Simpson index decreased in the MG group, which indicates that high-altitude hypoxia exposure increased the diversity of intestinal flora. The Shannon and Simpson indexes after irbesartan intervention were close to the levels of the CG group; thus, irbesartan can return the diversity of intestinal flora to a normal status.

A PCA of the bacterial community was performed as shown in Figure 7A. The clear separation between the two clusters, which represent the microbial composition of CG and MG, indicates that the two intestinal environments were completely different, and the IB.H group was closer to CG compared with MG. The results showed that 32.52% and 20.91% of the total variations were explained by the first two principal components, respectively. Thus, a substantial difference exists

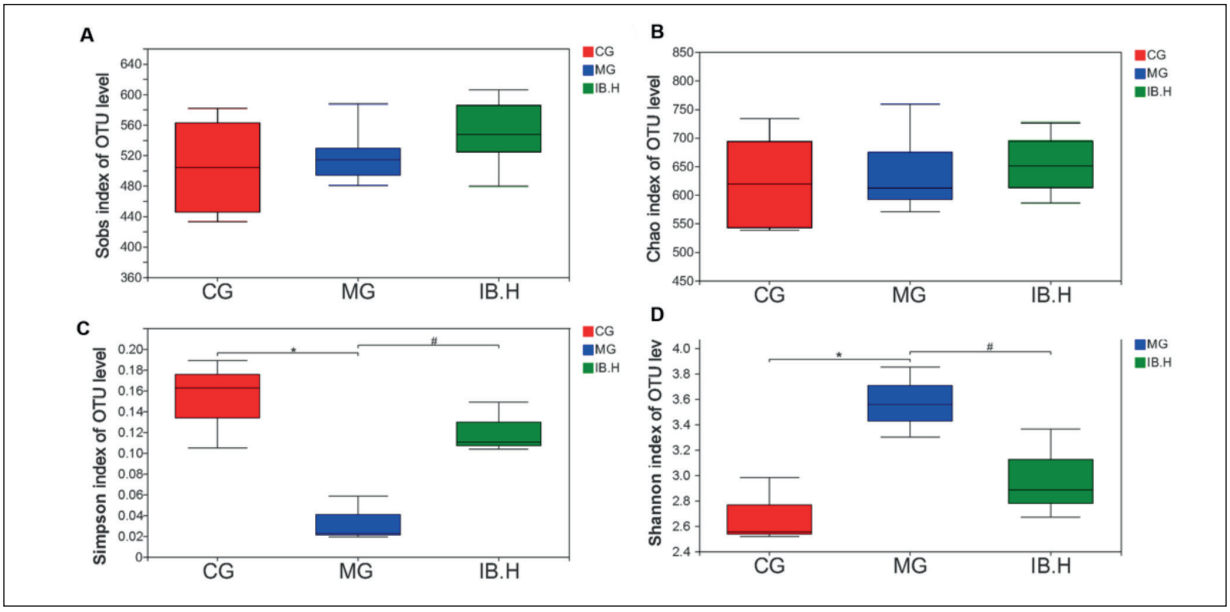

Figure 6. Boxplots of alpha diversity measures of colonic microbiota from each group. **A,** Species observed. **B,** Chao diversity index. **C,** Simpson diversity index. **D,** Shannon diversity index. CG means control group (red box). MG means HAPH model group (blue box). IB.H means MG group treated with 27 mg/kg·day irbesartan (green box). n = 10 per group. Differences among groups were calculated using ANOVA followed by Tukey's post-hoc test. *Means significant difference (p<0.05) compared with CG rat by Student's t-test. # means significant difference (p<0.05) compared with MG model rat by Student's t-test.

between the MG and CG groups, and the flora structure after irbesartan administration tends to be similar to that in the CG group.

Partial least squares discriminant analysis (PLS-DA) processes multivariate data. The principle of PLS-DA is the same as that of PCA, but the data structure is projected by partial least squares method. PLS-DA can reduce the impact of multicollinearity between variables and is very suitable for the analysis of microbial community diversity. Figure 7B shows that the flora structures in the MG group and irbesartan treatment groups were substantially different from the CG group.

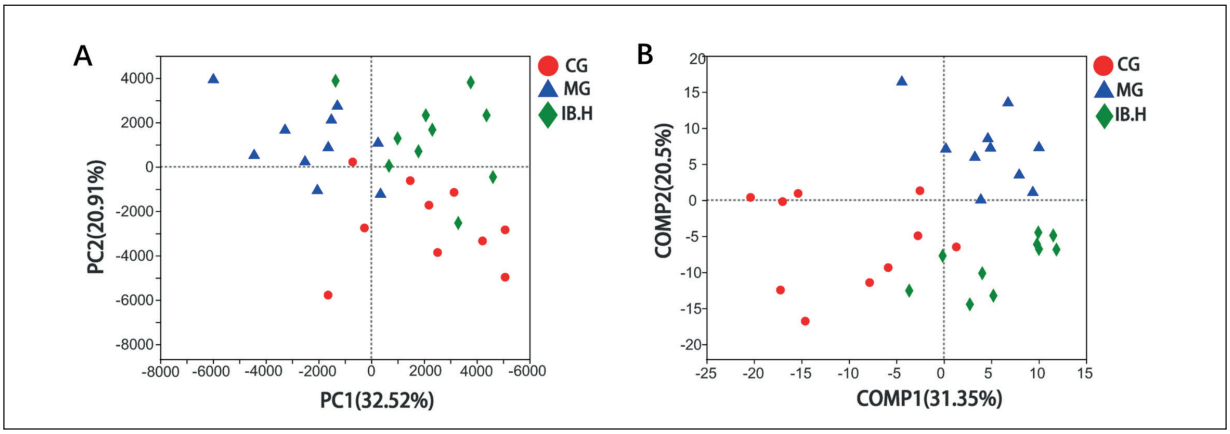

Figure 7. PCA and PLS-DA score plots. **A,** PCA score plot. Unweighted UniFrac separates the control group (CG), HAPH model group (MG), and high-dose irbesartan group (IB.H). The horizontal ordinate indicates the score of samples in the first principal component, and the vertical ordinate indicates the score of samples in the second principal component. **B,** PLS-DA score plot. COMP1, the interpretable degree of the first principal component; COMP2, the interpretable degree of the second principal component. PLS-DA was conducted to compare the overall differences in OTU profiles among the control group (CG), HAPH model group (MG), and high-dose irbesartan (27 mg/kg·day) group (IB.H).

Changes in Intestinal Flora at the Phylum and Family Levels

The analysis of the phylum composition of the bacterial flora in the intestinal contents of rats shows that *Firmicutes* was the most abundant phylum, followed by *Bacteroides*, *Actinomyces*, and *Proteobacteria* (Figure 8A). The proportion of *Firmicutes* in the MG group was substantially higher than those in the CG and IB.H groups, whereas the proportion of *Bacteroides* was lower than those in the other two groups. Irbesartan treatment restored *Firmicutes* and *Bacteroides* to similar levels in CG. In the family level, the abundance of *Prevotellaceae* and *Desulfovibrionaceae* increased whereas the abundance of *Lactobacillaceae* and *Lachnospiraceae* decreased in the intestines of rats in the MG group. The abundance of these florae was restored or partially restored to normal levels after irbesartan intervention. The *Firmicutes*-to-*Bacteriodetes* (F/B) ratio of the MG group was 1.23 times higher than that of the CG group and returned to normal level by irbesartan intervention (Figure 8C).

Discussion

In this study, we first demonstrated that irbesartan can ameliorate pulmonary artery pressure, left ventricular systolic dysfunction, cardiomyopathy, and the release of vasoconstrictor factors and inflammatory mediators caused by chronic hypoxia exposure. Further, we first found that disorders in oxidative stress and intestinal flora in HAPH can be reversed by irbesartan. The model of chronic mountain sickness can be established by simulating the plateau environment, and its main pathological features are high-altitude polycythemia (HAPE) and HAPH. Most research on the differential spectrum of intestinal flora are based on normal people who live in high-altitude areas; however, the intestinal flora of HAPH has not been reported yet. Chronic hypoxia induces the synthesis of ACE in pulmonary vascular endothelial cells. ACE converts angiotensin I (AngI) to AngII and leads to the degradation of the vasodilatation material, resulting in the increase of pulmonary vasoconstriction and pulmonary vascular resistance, pulmonary arterial smooth muscle proliferation,

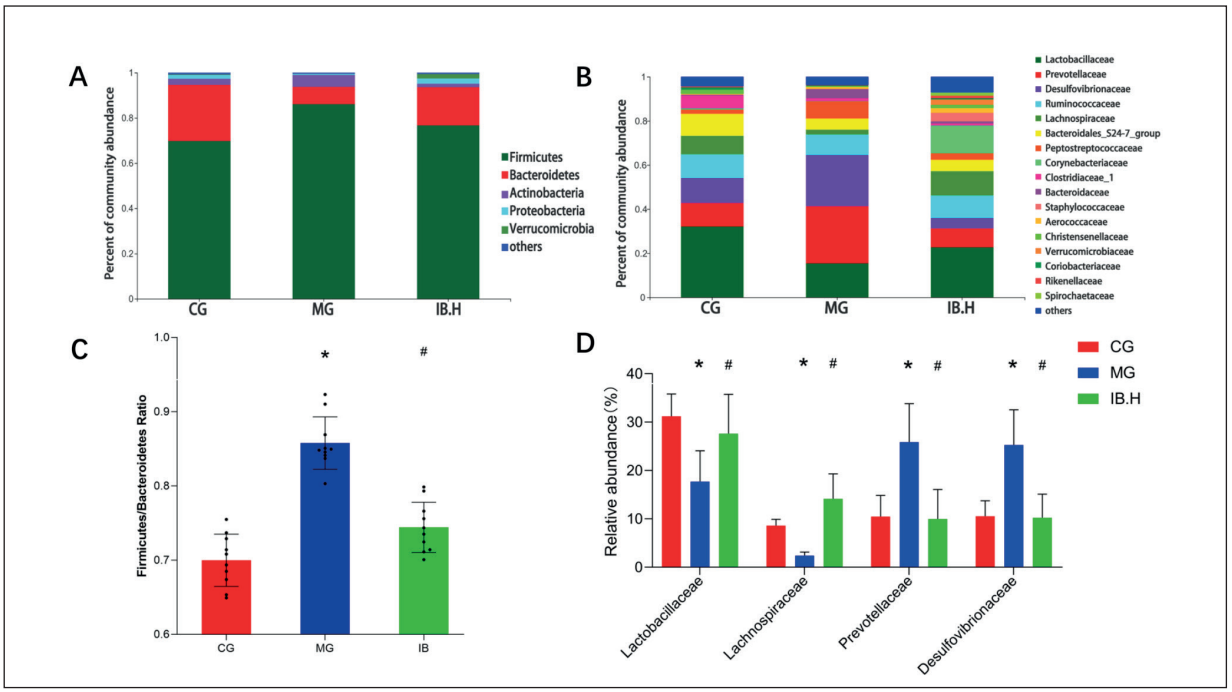

Figure 8. Effects of irbesartan on the bacterial flora composition of the intestinal contents of HAPH rats. **A,** Relative abundance of OTUs in bacterial phylum level. **B,** *Firmicutes/Bacteroidetes* ratio. **C,** Relative abundance of OTUs in bacterial family level. **D,** Relative abundance of *Lactobacillaceae, Lachnospiraceae, Prevotellaceae*, and *Desulfovibrionaceae*. Data are expressed as mean ± SD (n = 10 per group). Differences among groups were calculated using ANOVA followed by Tukey's post hoc test. *Means significant difference (p<0.05) compared with CG rat by Student's t-test. #means significant difference (p<0.05) compared with MG model rat by Student's t-test.

pulmonary vascular remodeling, and pulmonary hypertension; eventually, these processes lead to cardiac dysfunction[19]. AngII has a wide range of effects on the cardiovascular system, including the direct activation of AngII receptors present on vascular smooth muscle cells.

The results of our study showed that pulmonary arterial pressure was considerably increased in the high-altitude hypoxia rat model; thus, we successfully established the HAPH model. Further investigations through the pathological sectioning of cardiac muscles showed that the myocardial striations were unclear and myocardial interstitial vessels were remarkably congested. In addition, partial myocardial swelling and cytoplasm granule degeneration were observed, and eosinophilic degenerations on some myocardial fibers were significant. This evidence further strengthens the notion that our hypoxia rat model was established successfully. The results also clearly indicate that high-altitude hypoxia may affect the morphology of cardiac muscles. The echocardiography of the model rats revealed different degrees of narrowing of the left ventricular systolic and diastolic diameters. Typical pulmonary hypertension was observed. The syntropy movements of the interventricular septum and the left ventricular posterior wall demonstrate the effect of pulmonary hypertension on the left ventricular systolic and diastolic functions. Therefore, AngII plays a key role in the cardiac dysfunction caused by chronic hypoxia. Irbesartan is an antagonist of AngII receptors (e.g., AngII type 1 receptor [AT1R]) and blocks the connection between the AngII overexpressed by chronic hypoxia and AT1R at the final stage; therefore, it inhibits the physiological effect of AngII, relieves the intense contraction of blood vessels, reduces pulmonary hypertension, and prevents and reverses cardiovascular remodeling. Pulmonary artery pressure declined after irbesartan intervention compared with the model group. The myocardial fibers lined up in orderly rows, and only mild congestion was observed in some of the myocardial interstitium. The echocardiography showed that irbesartan treatment was effective in improving left ventricular diastolic dysfunction, and high-dose irbesartan treatment was remarkably effective.

Oxidative stress and systemic inflammatory response may be the two main factors of cell damage caused by hypobaric hypoxia. Cell energy production is hindered and a large amount of oxygen free radicals are produced during hypoxia. AngII can also promote the production of oxygen free radicals[20]. Excessive oxygen free radicals consume SOD and lead to cell oxidative damage. As a consequence, endothelium-dependent vasodilation response is weakened, and vasoconstriction response is enhanced[21]. The test results of oxidative stress indicators in this experiment showed that compared with the CG group, the MG group had increased oxygen free radicals in intestinal tissues, decreased antioxidants, unbalanced oxidation and antioxidant effects, and oxidative stress. However, irbesartan can reverse the oxidative stress caused by low pressure and hypoxia; therefore, irbesartan can alleviate the intestinal oxidative damage caused by hypoxia and protect the shielding function of the intestine.

High-altitude hypobaric hypoxia, as an environmental stressor, breaks the balance between pulmonary vasoconstriction and relaxation and ultimately forms pulmonary hypertension. AngII, ET-1, and NO are important vasodilation regulators[22,23]. AngII leads to pulmonary artery smooth muscle cell proliferation through AT1R, which is one of the reasons for pulmonary artery remodeling[24]. Under normal physiological conditions, ET-1 can stimulate the release of endothelial NO and therefore reduce its vasoconstriction effect. ET-1 and NO are in a dynamic equilibrium relationship in the body[25]. ET-1 increases remarkably under hypobaric hypoxia[26], and endogenous NO synthesis is reduced[27]. Once the ET-1/NO balance is broken, the vasoconstriction and relaxation of blood vessels will be impeded, pulmonary artery smooth muscle relaxation response will be decreased, and pulmonary artery pressure will increase. The test results of vasoactive substances in this experiment showed that the simulated high-altitude environment can stimulate the increased release of vasoconstrictor substances and the decreased release of vasodilator substances, which lead to pulmonary hypertension. Irbesartan can adjust the balance between ET-1 and NO, maintain the normal function of blood vessels, and play a role in reducing pulmonary artery pressure, which may be achieved by blocking the biological effects of AngII.

High-altitude hypobaric hypoxia leads to systemic inflammatory response, which is caused by inflammatory chemokines[9]. IL-6 and CRP are reliable and sensitive markers of systemic inflammation and represent the occurrence of systemic inflammation in a plateau environment[9]. CRP is one of the acute-phase response proteins and an inflammatory marker of tissue damage[28]. AngII

can induce CRP[29] and IL-6[30]; thus, AngII increased by hypoxia promotes inflammation in the body. Inflammation medium can induce the proliferation and differentiation of pulmonary artery endothelial cells, smooth muscle cells, and fibroblast and the production of matrix protein; promote the reactivity of vascular active substances and the infiltration of inflammatory cells; mediate vascular inflammation; activate fibroblast and smooth muscle cells in the outer membrane to secrete chemotaxis factors to participate in the process of vascular remodeling, all of which lead to pulmonary hypertension[31]. The test results of inflammatory markers in this experiment showed that the levels of CRP and IL-6 in the serum of rats in the MG group were remarkably increased, and the myocardial pathological sections also showed that myocardial swelling and inflammatory cell infiltration occurred in the plateau group. These findings suggest a strong inflammatory response in rats. Compared with those in the MG group, the CRP and IL-6 contents in the serum of rats were reduced, the myocardium was arranged regularly, and the inflammatory cell infiltration disappeared after irbesartan administration. Thus, irbesartan has certain anti-inflammatory activity in the body. Angiotensin receptor blockers have intestinal anti-inflammatory properties by inhibiting the AT1R axis[32]. Therefore, the alleviation of HAPH symptoms was likely achieved by the anti-inflammatory activity of irbesartan.

Recently, intestinal flora at high altitude has gradually become a concern in clinical research. Poor acclimatization to high altitude hypoxia environment may cause reformation in intestinal flora, which is induced by alteration in immunity because of low oxygen partial pressure[33]. Long-term exposure to high altitude hypoxia results in remarkable differences in the composition and diversity of intestinal flora compared with humans from the plain[34]. However, changes in the composition and diversity of intestinal flora in HAPH have not yet been reported. The balance and overall stability of the microbial community ecosystem are considered the key factors for maintaining health. However, many external factors can change intestinal microbial community while inhibiting beneficial bacteria and thus creating conditions for the growth of harmful bacteria and lead to the imbalance of intestinal flora. Evidence shows that intestinal disorders and high blood pressure are closely related[35]. The research results suggest that the number of intestinal florae did not change substantially with the increase in low pressure and hypoxia but was affected by the increase in the richness and diversity of intestinal flora. For example, Sob and Chao index, which represent the number of flora and species abundance in the altitude sickness group, were not substantially different; the Shannon index decreased; and the Simpson index increased during hypoxia. Shannon and Simpson indexes recovered or were close to normal levels after irbesartan intervention. Chronic hypoxia leads to the gradient drop of partial oxygen pressure in the intestinal lumen, which results in a decrease in the relative abundance of aerobic bacteria. However, obligate and facultative anaerobic bacteria appeared; therefore, the number of florae was basically unchanged, but their diversity increased.

The F/B ratio is a sign of intestinal dysfunction in patients with pulmonary hypertension. Changes in the composition of intestinal microbiota are related to the integrity of the intestine, and the destruction of this barrier is more conducive to the growth of aerobic bacteria[36]. The antihypertensive drug candesartan can protect the hypertension-associated intestinal barrier by increasing the production of short-chain fatty acids (SCFAs) by microorganisms[37]. ACE inhibitors enalapril and captopril can protect the integrity of the intestinal barrier in hypertensive rats while controlling blood pressure[38]. Our research results showed that the F/B ratio increased in the simulated plateau environment and was also close to that of the CG group after irbesartan treatment; thus, the F/B ratio can be a prognostic marker in a series of diseases caused by chronic hypoxia at high altitude. Our results are similar to those in a study of obstructive apnea syndrome (OSAS), that is, chronic hypoxia can lead to changes in the overall distribution of intestinal flora as manifested by the increased F/B ratio and Shannon index. PCA after hypoxia is also substantially different from normal controls[39]. This result indicates that the main cause of changes in the intestinal flora in HAPH may be exposure to chronic hypoxia.

In the family level, more *Prevotellaceae* and *Desulfovibrionaceae* were present in the intestines of rats in the MG group, whereas the abundance of *Lactobacillaceae* and *Lachnospiraceae* decreased. These fungi can be restored or partially restored to normal levels after irbesartan intervention. *Prevotellaceae* produces endotoxin and encodes superoxide reductase, which is easily transported through the barrier into the systemic circulation and leads to inflammation. In addition, *Prevotellaceae* and *Desulfovibrionaceae* can lead

to mucoprotein degradation, that is, the sulfate released when *Prevotellaceae* degrades mucoprotein is removed by *Desulfovibrionaceae* bacteria. Such process further promotes the degradation of mucoprotein and therefore destroys intestinal wall integrity and permeability[40]. *Lactobacillaceae* is anti-inflammatory and can increase serum SOD activity, reduce MDA content, inhibit lipid peroxidation, and increase reactive oxygen species clearance to protect tissues[41]. *Lactobacillaceae* population damage is associated with the pathological process of hypertension. Candesartan (an AT1R inhibitor) treatment can substantially increase the content of *Lactobacillaceae*[37]. Cohort studies have shown that beneficial SCFA-producing bacteria are reduced in patients with pulmonary hypertension. For example, the reduction of *Lachnospiraceae* can increase intestinal permeability[42]. *Lachnospiraceae* can produce butyric acid through the butyryl-CoA, acetate-CoA transferase, or butyrate kinase pathway by utilizing lactic acid and acetic acid, which exert a protective effect on intestinal shielding[43]. *Lachnospiraceae* is also negatively related to the contents of IL-6 and other inflammatory factors in the body[44]. The abundance of *Lachnospiraceae* is decreased whereas the abundance of *Prevotellaceae* is increased in OSAS[39,45]. These findings are similar to our research results. The decreased abundance of probiotics, such as *Lactobacillaceae* and *Lachnospiraceae*, and the increased abundance of harmful bacteria, such as *Prevotellaceae* and *Desulfovibrionaceae*, are speculated to be caused by chronic hypoxia. Metabolites in the body that are changed with the imbalance of florae may damage intestinal epithelial tissue, destroy the tight junctions between cells, and produce harmful substances, such as endotoxins, which enter the blood circulation through the damaged intestinal barrier and cause inflammation. Therefore, cardiovascular disease occurs. Irbesartan and other angiotensin blockers act by antagonizing AngII[37,38]. Thus, we speculated that the mechanism of irbesartan's resistance to HAPH may be acted through intestinal barrier damage, SCFA secretion imbalance, and the decrease in the number of intestinal probiotics. However, further verification is needed.

Conclusions

We found for the first time that irbesartan treatment can improve intestinal disorders in rats under low pressure and hypoxia. Irbesartan, an AngII receptor blocker, can improve the effects of simulated high-altitude hypobaric hypoxia in rats. Its mechanism may be explained as follows. 1) Irbesartan improves intestinal integrity and defensin production; restores the changes in intestinal microbiota caused by hypoxia; helps protect blood vessels, especially the pulmonary artery; and reduces pulmonary hypertension caused by hypoxia by regulating the body's immune system at least to a certain extent. 2) Irbesartan directly blocks the binding of AngII to its receptor (AT1R); thus, the body's high response state is improved by increasing the NO content in the circulation, inhibiting vasoconstriction, and reducing pulmonary artery pressure[46]. 3) Irbesartan reduces the level of oxidative stress mediated by the combination of AngII and AT1R by downregulating systemic inflammatory response. Hypoxia destroys the integrity of the intestine and increases the level of intestinal oxidative stress. The metabolites of intestinal florae enter the blood circulation and promote inflammatory response throughout the body. By contrast, irbesartan restores the diversity of florae to a normal level apart from inhibiting the inflammation and reducing the production of vasoactive substances; therefore, it reduces the entry of metabolites into the bloodstream and ultimately achieves anti-inflammatory effects.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics statement

The animal studies were conducted in accordance with the Chinese Laboratory Animal Administration Act of 2017. All experiments that involved animals and the protocols for the use of samples from rats were approved by the Animal Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Approval number: IACUC-20190225-70).

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