

The Effect of HIF1- α /NF- κ B/MMP2 Signaling Pathways Mediated by ALDH2 on Chronic Intermittent Hypoxia Induced Myocardial Injury in Rats

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Abstract: (1) Objective: To investigate the effect of HIF1- α /NF- κ B/MMP2 signaling pathways mediated by ALDH2 on chronic intermittent hypoxia induced myocardial injury in rats. (2) Methods: Thirty SD rats were randomly assigned to three groups (10 rats in each group): control group (CONTROL), chronic intermittent hypoxia group (CIH), and CIH + ALDH2 group. Rats in the CIH + ALDH2 group were established by an intraperitoneal injection of the ALDH2 activator, Alda-1 (20 mg/kg), once a day for three consecutive days. After three months, changes in ventricular function were determined by ultrasound. The expressions of HIF1- α , NF- κ B, and MMP2 proteins were detected by protein blotting, and the concentration of 4-HNE in myocardial tissue was measured. (3) Results: Compared with the control group, echocardiography showed that rats in the CIH group had significantly reduced myocardial function, elevated protein levels of HIF1- α , NF- κ B, and MMP2 in myocardial tissues, and increased the expression of 4-HNE in myocardial tissues ($P < 0.01$); Compared with the CIH group, rats in the CIH + ALDH2 group showed significant improvement in myocardial function, decreased protein levels of HIF1- α , NF- κ B, and MMP2 in myocardial tissue, and decreased the expression of 4-HNE in myocardial tissue ($P < 0.01$). (4) Conclusion: Chronic intermittent hypoxia increases the expression of HIF1- α to damage the myocardium and affect cardiac function, and ALDH2 inhibits the expression of HIF1- α to protect the myocardium.

Keywords: Myocardial Injury; Chronic Intermittent Hypoxia; ALDH2; HIF1- α ; NF- κ B; MMP2.

1. Introduction

Obstructive Sleep Apnea Hypopnea Syndrome (OSAHS) is characterized by recurrent complete and partial upper airway obstructive events, resulting in intermittent hypoxemia, autonomic fluctuation, and sleep fragmentation. OSA prevalence is as high as 40% to 80% in patients with hypertension, heart failure, coronary artery disease, pulmonary hypertension, atrial fibrillation, and stroke [1]. The impaired gas exchange caused by OSAHS leads to chronic intermittent hypoxia (CIH) in tissues, which is a major cause of systemic injury and an independent risk factor for cardiovascular disease, and its mechanisms include oxidative stress, inflammatory response, and endothelial dysfunction [2]. Clinical studies have shown that hypoxia-inducible factor-1 (HIF-1) levels in organs, tissues, and circulating blood of OSAHS patients are significantly higher than those of normal subjects, and that the occurrence of dysbiosis in OSA patients may be mainly mediated by the α -subunit of HIF-1 [3]. HIF-1 is a heterodimeric complex consisting of HIF-1 α and HIF-1 β subunits. Animal experiments have shown that HIF-1 α transcriptional activation promotes cardiomyocyte hypertrophy [4]. Under hypoxia, the expression of HIF-1 α is elevated and translocated into the nucleus to convert into HIF-1, which binds to various target genes in the nucleus to generate a hypoxia-induced response and promotes the transduction of many signaling pathways [5]. HIF, with the assistance of the hypoxia-responsive promoter, induces the transcription of the nuclear factor-kappa B (NF- κ B), matrix metalloproteinase 2 (MMP2) [6, 7], and regulates the expressions of reactive oxygen species (ROS) and 4-hydroxy-2-nonenal (4-HNE) [8].

Aldehyde dehydrogenase 2 (ALDH2) is an important aldehyde oxidase in mitochondria. ALDH2 inhibits the proliferation of cardiac fibroblasts in high glucose environments, reduces the protein expression of ROS and 4-HNE, and decreases oxidative stress as well as collagen expression, reversing myocardial fibrosis and attenuating ischemia and reperfusion injury [9, 10].

This study aimed to investigate the effects of ALDH2 on hypoxic myocardium and HIF-1 α pathway by preparing a myocardial injury model in CIH rats.

2. Materials and Methods

2.1. Reagents and Instruments

Alda 1 (Sigma Aldrich); ECL luminescence detection kit (Millipore); ELISA kit (Shanghai Zhenke Biotechnology); Sheep anti-mouse secondary antibody (Wuhan PhD Bioengineering); Sheep anti-HIF1- α , NF- κ B, MMP2, β -actin monoclonal antibodies (santa cruz).

2.2. Animals

Thirty male SD rats were obtained from the Animal Experiment Center of Bengbu Medical College. The rats were randomly assigned to three groups (10 rats in each group): control group (CONTROL), chronic intermittent hypoxia group (CIH), and CIH + ALDH2 group.

2.3. Animal Model Preparation

Rats in the CIH group, and CIH + ALDH2 group were placed in containers with chronic intermittent hypoxia, and rats in the CONTROL group were placed in normal oxygen supply containers of the same size. The chronic intermittent hypoxia setup: The oxygen concentration in the vessel

decreases to 4-5% for 20 seconds, then air is introduced to adjust the oxygen concentration upward to 21% for 40 seconds. This cycle was maintained for eight hours per day. Rats in the CIH + ALDH2 group were established by an intraperitoneal injection of the ALDH2 activator, Alda-1 (20 mg/kg), once a day for three consecutive days.

2.4. Measurement of Myocardial Function in Rats

Atrial and ventricular internal diameters were measured based on QRS wave clusters, T-waves, and opening and closing of the mitral valve. The left intraventricular diameters and left atrial diameters of the rat heart were measured in the left heart long-axis section; the ejection fraction and the left intraventricular diameter shortening were obtained by M-mode ultrasound. All data were averaged over three measurements.

2.5. Myocardial Expressions of HIF1- α , NF- κ B and MMP2 Protein Detected by Western Blotting

100 mg of heart tissue per rat was placed in a 2.0-mL centrifuge tube, and 1 mL PIPA cracking liquid with 10 μ L PMSF inhibitors was added to the tube. Then, the mixture was centrifuged at 16,000 g/min for 5 min. The liquid layer, which contained the total protein, was saved, and transferred to a new tube. The protein concentration was detected with the Total Protein Assay kit according to the manufacturer's instructions. The total protein solutions were adjusted to the same concentration (10 μ g/ μ L) with 5 $\times$ SDS sample buffer, which was eventually dissolved to 1 $\times$ SDS. Finally, the protein samples were boiled for 10 minutes and saved at -20°C. 60 μ g total protein was added to each well and separated via SDS-PAGE. Then, the separated proteins were transferred to a Nitrocellulose membrane. Next, the membranes were blocked for 2 h with 5% skim milk powder diluted in TBST (Tris Buffered Saline with Tween-20) at room temperature, followed by incubation with primary antibodies for 12 h and then secondary antibodies for 2 h. The primary and secondary antibodies were used in the Western-Blot experiments. Then, the membranes were washed with TBST for 30 min, followed by detection with chemiluminescence and image densitometry analysis.

2.6. Determination of 4-HNE in Rat Myocardial Tissue

0.1g of myocardial tissue was taken and homogenized in chilled PBS buffer. 4-HNE was processed according to the kit instructions and a standard curve was drawn to calculate the expression level of the myocardial tissue samples.

2.7. Statistical Methods

SPSS 22.0 data statistics software was used to analyze the data and statistics, and the measurements were expressed as mean and standard deviation (xs). One-way ANOVA was applied between groups; $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1. Comparison of Myocardial Function in Rats

The data showed that LVIDd and LVIDs of rats in the CIH group were significantly higher than those in the Control group, whereas the opposite was true for EF and FS ($P < 0.01$). The levels of LVIDd and LVIDs were decreased in the CIH + ALDH2 group compared with the CIH group, along with an increase in EF, FS ($P < 0.01$, Fig. 1).

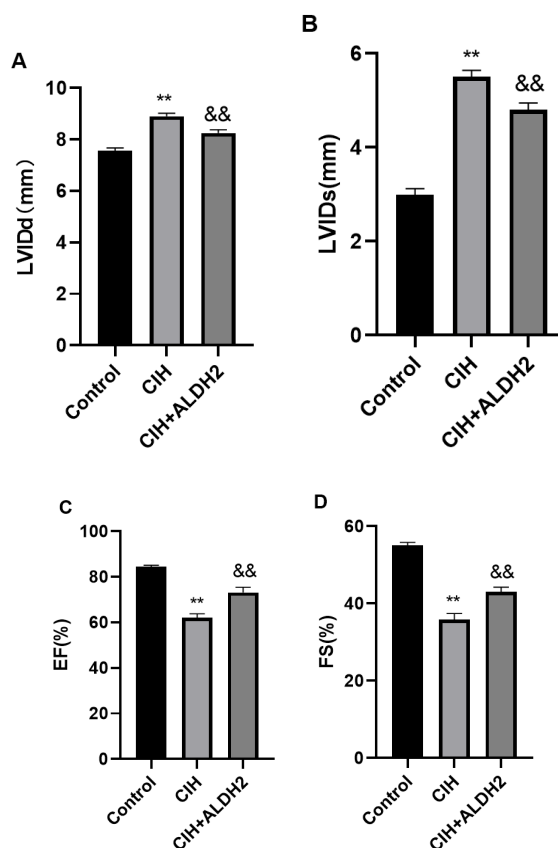


Figure 1. Comparison of myocardial function in rats (Mean $\pm$ SD, n=10)

Note: ** $P < 0.01$ vs control group, && $P < 0.01$ vs CIH group.

3.2. Myocardial Expressions of HIF1- α , NF- κ B and MMP2 Protein

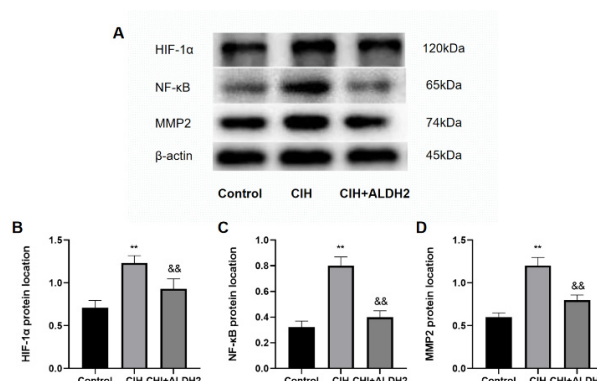


Figure 2. Myocardial expressions of HIF1- α , NF- κ B and MMP2 protein detected by Western blotting (A) and quantitative analysis of their protein expressions (B, C, D) in different groups (Mean $\pm$ SD, n=10)

Note: ** $P < 0.01$ vs control group, && $P < 0.01$ vs CIH group

The expressions of HIF1- α , NF- κ B, and MMP2 proteins in the CIH group were higher than that in the Control group ($P<0.01$); the expressions of HIF1- α , NF- κ B, and MMP2 proteins in the CIH + ALDH2 group was lower than that in the CIH group ($P<0.01$, Fig. 2).

3.3. Levels of Myocardial 4-HNE

The level of 4-HNE in the myocardial tissues of rats in the CIH group was higher than that in the Control group ($P<0.01$); the level of 4-HNE in the myocardial tissues of rats in the CIH + ALDH2 group was lower than that in the CIH group ($P<0.01$, Table.1).

Table 1. Levels of myocardial 4-HNE in different groups (Mean $\pm$ SD, n=10)

Group	4-HNE
Control	5.63 $\pm$ 0.27
CIH	45.07 $\pm$ 5.88 **
CIH+ALDH2	27.7 $\pm$ 6.05 &&

Note: ** $P<0.01$ vs control group, && $P<0.01$ vs CIH group.

4. Discussion

The prevalence of OSAHS among cardiac patients is as high as 40%-80%, and cardiac patients are more susceptible to adverse cardiovascular events caused by OSAHS. Still, the rate of knowledge and rational treatment of OSAHS is low[1]. Continuous positive intra-airway pressure ventilation is the gold standard for the treatment of OSAHS, but some studies have concluded that the former does not improve the prognosis and mortality in patients with OSAHS combined with heart disease[11]. Finding new targets to ameliorate myocardial injury due to OSAHS is a current research hotspot. The impaired gas exchange caused by OSAHS leads to intermittent tissue hypoxia, which elevates patients' nocturnal systolic and 24-hour diastolic blood pressures[12], increases left ventricular afterload and vascular transmural pressures, leading to increased ventricular wall tension and myocardial oxygen consumption, impaired left ventricular diastolic function, and further impediments to left ventricular filling. This leads to a decrease in per-pulse output and cardiac output, and ultimately to cardiac dysfunction. We also found that CIH induced a functional decline in LV systolic and diastolic function in rats.

Clinical studies have shown that the expression of HIF-1 is increased in patients with OSAHS[3], and different studies have come to different conclusions about whether the expression of HIF-1 α is detrimental to the myocardium. Marion et al. found that sustained upregulation of HIF-1 α -expression accelerated cardiac dysfunction in rats[13]. However, Sano et al. found that HIF-1 α promotes cardiac vascularization and prevents cardiac hypertrophy and contractile dysfunction from occurring[14]. Our study showed that three months of CIH resulted in high HIF-1 α -expression and a decrease in LV systolic function, which is consistent with the results of a national study[5], which suggested that short-term HIF-1 α upregulation provides some benefits, while long-term HIF-1 α high expression over eight weeks eventually leads to myocardial damage in rats. The expression of HIF-1 α is elevated under hypoxia and translocates into the nucleus to be converted into HIF-1, which binds to various target genes in the nucleus to generate a hypoxia-induced response and promote the transduction of multiple signaling pathways[5]. Wei et al. found that the expression levels of HIF-1 α , NF- κ B, IL-6, and MMP2 were

significantly elevated in the state of CIH, which triggered apoptosis, myocardial fibrosis, and diminished cardiac ejection capacity[15]. HIF-1 α induces NF- κ B production under hypoxia, which acts together with ROS to enhance the inflammatory response to damaged cells and tissues[16, 17]. Wang et al[7]found that a protein could contribute to the elevation of the expressions of ROS and HIF-1 α , which in turn up-regulated MMP2, enhanced the myocardial oxidative stress response process, and exacerbated myocardial ischemia-reperfusion injury. Studies on lung tissues have shown that inhibiting the expression of HIF-1 α and ROS in a hypoxic environment reduces the levels of inflammatory factors IL-1 β , TNF- α , and IL-6[18]. Our study showed that CIH increased the expression of HIF-1 α , along with a significant increase in NF- κ B, MMP2, and 4-HNE levels.

Mitochondria are the primary site of cellular energy production and oxidative phosphorylation, and are involved in the regulation of calcium signaling, cell proliferation, and metabolism; mitochondria are closely related to cellular hypoxia receptors and play an important role in the pathogenesis of OSAHS. Electron leakage that occurs during the production of ATP by the mitochondrial electron respiration chain is the main cause of ROS production. Normally, endogenous antioxidant molecules can counteract the effects of ROS under intermittent hypoxia. Still, the prolonged presence of intermittent hypoxia in OSAHS patients results in persistently high levels of intracellular ROS, leading to mitochondrial dysfunction and exacerbating the process of cardiac fibrosis in patients with OSAHS. The protective effect of ALDH2, an essential aldehyde oxidase in the mitochondria against myocardial injury, has gained increasing consensus. ALDH2-deficient formaldehyde-treated rats increased the expression of HIF1- α in macrophages, contributing to enhanced inflammatory responses and glycolysis [19]. Our study found that ALDH2 resulted in a decrease in the expression of HIF-1 α , along with significant reductions in NF- κ B, 4-HNE, and MMP2 levels, and attenuation of left ventricular systolic dysfunction and cardiac remodeling. Our previous study found that ALDH2 plays an important role in antagonizing the formation of ROS, attenuating their cellular damage, and mitigating oxidative stress, among other pathophysiological processes [20]. ALDH2 inhibits the proliferation of cardiac fibroblasts in the high-glucose milieu, reduces the expression and release of ROS and 4-HNE proteins, and reduces the oxidative stress overload as well as the expression of collagen I and collagen III, and reverses myocardial fibrosis [10, 21]. Hua et al[22]found that the ALDH2 agonist Alda-1 reduced myocardial weight/body weight ratio, collagen volume, cardiomyocyte apoptotic index, end-diastolic left ventricular internal diameter, and end-systolic left ventricular internal diameter in myocardial infarcted rats, as well as increased left ventricular ejection fraction and that its protective effects were associated with a reduction in the accumulation of 4-HNE, and that Alda-1's long-term treatment prevented the progression of ventricular remodeling.

In conclusion, ALDH2 inhibits impaired cardiac function caused by CIH, and its mechanism of action may be to inhibit smooth muscle cell dedifferentiation by regulating the HIF1- α /NF- κ B/MMP2 pathway, which provides a new strategy and a new basis for the prevention and treatment of OSAHS.

Acknowledgments

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