Influence of the TLR4-mediated p38MAPK signaling pathway on chronic intermittent hypoxic-induced rat's oxidative stress and inflammatory cytokines in rats

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Abstract. – OBJECTIVE: To investigate the influence of the TOLL-like receptor 4 (TLR4)-mediated p38MAPK signaling pathway on chronic intermittent hypoxic (CIH)-induced oxidative stress and inflammatory cytokines in rats.

MATERIALS AND METHODS: A total of 120 healthy male Sprague Dawley (SD) rats, aged between 8-10 weeks, were randomly divided into 9 groups (normoxia control group, CIH 2 weeks group, CIH 4 weeks group, CIH 6 weeks group, CIH 8 weeks group, CIH 6 weeks + p38MAPK receptor inhibit group, CIH 6 weeks + Tempol group, CIH 8 weeks + p38MAPK receptor inhibitor group and CIH 8 weeks + Tempol group). The expression of TLR4 and p38MAPK in the adipose tissue was evaluated, as well as the level of serum oxidative stress markers (SOD, TRx-1, MDA) and inflammatory cytokines (adiponectin, TNF-α, hsCRP and IL-6). RT-PCR and Western-blot were conducted to detect the expression of TLR4 and p38MAPK mRNA.

RESULTS: With increased hypoxia, the levels of SOD and adiponectin in the serum of the CIH group decreased significantly, and the levels of TNF-α, hsCRP, IL-8 and IL-6 in serum increased significantly. After the intervention of antioxidant Tempol and p38MAPK inhibitor SB203580, SOD increased significantly but with significant MDA reduction. The levels of TNF-α, hsCRP, IL-8 and IL-6 in serum significantly decreased. The results of RT-PCR and Western-Blot indicated that the P-p38 and TLR4 proteins related to the MAPK pathway were expressed in rat adipose tissue. With the hypoxia intensity, expression of P-p38 decreased after initially increasing. The expression of TLR4 showed a continuously growing trend. After Tempol treatment, the expression of p38MAPK protein decreased, and the expression of TLR4 did not change significantly, indicating the inhibiting effect of Tempol on p38MAPK, without a significant inhibiting effect on TLR4.

CONCLUSIONS: The TLR4-mediated p38MAPK signaling pathway was active in adipose tissue and the expression of the corresponding molecules increased as the duration of intermittent hypoxia increased. The expression of TLR4 and p38MAPK components regulated the level of oxidative stress and inflammatory cytokines; the application of p38MAPK inhibitors and antioxidant free radical scavengers improved the levels of oxidative stress and inflammatory cytokines.

Key Words
Obstructive sleep apnea syndrome (OSAS), TLR4, p38MAPK signaling pathway, Oxidative stress, Cytokines.

Introduction

Obstructive sleep apnea syndrome (OSAS) is a sleep-disordered breathing (SDB) disease characterized by recurrent upper airway collapse during sleep, which may be accompanied by the repeated occurrences of upper airway collapse at night, repeated hypoxia, or hypercapnia and sleep structure disorder[1]. Currently, clinical and animal experiments have proven that the chronic intermittent hypoxia (CIH) in OSAS may damage multiple organs, including heart, brain, kidney and lung[2]. Meanwhile, it is closely related to disorders of the metabolic endocrine system[2]. Therefore, OSAS is an independent risk factor for hypertension, coronary heart disease, diabetes mellitus and other common diseases[2]. It is proved that OSAS patients have oxidative stress, which is implicated as one of the important reasons for cardiovascular complications[3]. Even more, CIH is found associated with oxidative stress and inflammation[4]. During the oxidative stress process, the levels of SOD and reactive oxygen species may increase significantly, while the levels of inflammation factors, such as the tumor necrosis factor (TNF), interleukin (IL) and C-reactive protein (CRP), may also increase

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significantly during an inflammatory reaction. OSAS is accompanied by obesity and cardiovascular diseases, as well as confounding factors that may cause oxidative stress and inflammatory reaction. Currently, there are disputes regarding whether CIH, one of the core injury mechanisms of OSAS, can independently induce inflammation. Researches have shown that OSAS is an important risk factor resulting in insulin resistance, but independent of obesity. CIH caused by repeated upper airway obstruction, the main pathological characteristics of OSAS and the most important factor triggering systemic inflammation, plays a key role in the occurrence and development of metabolic dysfunction[5]. However, the precise mechanism by which intermittent hypoxia promotes metabolic disorders is unclear[6]. TOLL-like receptors (TLRs), a type of pattern recognition receptor and a inflammatory signaling portal protein, were first discovered in the human body in 1997 with more than ten variants discovered (TLR1-15) to date. TLR4, which is a member of the TLRs family commonly present in various tissues, can naturally identify lipopolysaccharide (LPS) and mediate natural immunity. TLR4 also plays an important role in regulating the immune reaction in noninfectious inflammatory diseases[7]. Upon activation, TLR4 can induce several signal transduction pathways in the cell and ultimately activate mitogen-activated protein kinase (MAPK) generate many proinflammatory factors that mediate the inflammatory reaction[8]. As an upstream regulatory factor regulating the expression of downstream MAPK signaling molecules, such as p38MAPK, TLR4 can regulate inflammatory reaction and also participate in insulin resistance and glycolipid metabolism in the MAPK pathway[9]. Therefore, the TLR4-mediated p38MAPK pathway may play a role in related metabolic diseases, such as OSAS, diabetes, and NASH. Although the CIH-simulated animal model of sleep apnea syndrome has been developed to study the mechanisms and its influence on endocrine metabolism[10], the occurrence and development of the TSL4-mediated p38MAPK pathway in OSAS oxidative stress and the inflammatory reaction are seldom evaluated. Tempol, which has an anti-hypoxia effect, may realize its mechanism by increasing SOD activity and reducing the contents of LDH and MDA[11]. Furthermore, its significant protective effect on hypoxic cells may realize its mechanism by removing the excessive ROS produced in the cells increasing the intracellular antioxidant ability, reducing the accumulation of lipid peroxidation products, and protecting the

completeness of cell membranes, increasing the expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF) mRNA and protein, reducing the expression of Caspase-3, lessening cell apoptosis[12]. The specific effects of high expression of VEGF, SOD and CAT on increasing the antioxidant capability, removing excess free radicals and reducing cell apoptosis still remain to be explored. Therefore, the aim of this research is to investigate the influence of the TLR4-mediated p38MAPK-signaling pathway on CIH-induced oxidative stress and inflammatory cytokines in rats, and evaluate the effect of Tempol in treating OSAS in the animal model.

Materials and Methods

Materials

A total of 120 healthy male Sprague Dawley (SD) rats weighing 180±20 g, aged between 8-10 weeks, were provided by Zhejiang University Animal Experimental Center and randomly divided into following nine groups: control group, CIH 2 weeks group, CIH 4 weeks group, CIH 6 weeks group, CIH 8 weeks group, CIH 6 weeks + p38MAPK receptor inhibitor group, CIH 6 weeks + Tempol group, CIH 8 weeks + p38MAPK receptor inhibitor group and CIH 8 weeks + Tempol group. There were 12 rats in each group. This study was approved by the Animal Ethics Committee of Huzhou Central Hospital Animal Center.

Reagent

A superoxide dismutase kit (SOD, Batch No.: 140927) and a malondialdehyde kit (MDA, Batch No.: 140923) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits IL-8β (Batch No.: 141202), IL-6 (Batch No.: 141128), and TNF-α (Batch No.: 141202) were produced by Shanghai ExCell Bio Company (Shanghai, China). The high-sensitivity C-reactive protein kit (hsCRP, Batch No.: 143025) was produced by Thermo Fisher Scientific (Waltham, MA, USA). Sodium dodecyl sulfate (SDS), ammonium persulfate (APS), acrylamide (Acrylamide), methylene bisacrylamide (BIS), tetramethyl diethylamine (TEMED), glycine (glycine), bromophenol blue (BPB), β-thioethanol (2-Me), and diethyl pyrocarbonate (DEPC) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). The total RNA extraction kit for animals and one-step fluorescence quantitative PCR kit were purchased from Beijing Tiangen Biochem-

ical Technology Co., Ltd., (Beijing, China). The p38MAPK inhibitor SB203580 (Batch No.: S1863) was purchased from Beyotime Biotechnology Company (Shanghai, China).

Instruments

A JXOC-12 low-pressure oxygen chamber automatic controller (Nanjing Xinfei Analytical Instrument Manufacturing Co., Ltd. Nanjing, China); sorting flow cytometer (Beckman Coulter Trading Co., Ltd. Pasadena, CA, USA); inverted phase contrast microscope (Shanghai Changfang Optical instrument Co., Ltd. Shanghai, China); micro-table high-speed centrifuge (Beckman Coulter Trading Co., Ltd., Brea, CA, USA); multi-function high-speed refrigerated centrifuge (Beckman Coulter Trading Co., Ltd. Brea, CA, USA); MLDEL680 microplate reader (Beijing Chengzhikewei Biotechnology Co., Ltd., Beijing, China); UV spectrophotometer (Shanghai Spectrum Instrument Co., Ltd., Shanghai, China); JS-780 automatic digital gel image analysis system (Shanghai Qingkai Experimental Equipment Co., Ltd., Shanghai, China); DYY-6D stabilized electrophoresis instrument (Shandong Biobase Group, Jinan, China) ;TE22 protein transmembrane system (Shanghai Flash Spectrum Biological Technology Co., Ltd., Shanghai, China); MINI-PROTEAN Tetra system (Bio-Rad, Hercules, CA, USA); Automatic Digital Imaging Analysis System Molecular Devices (Shanghai, China).

Methods

Sample Collection

(1) Serum collection: 8 rats were randomly selected from each group before the start of exposure, and at the 2nd, 4th, 6th, and 8th week after exposure. The serum of rats fasted for 10 h before the operation was collected and placed at room temperature for 2-3 h for centrifugation after precipitation at 3000 r/min for 10 min. Subsequently, 1 to 1.5 mL of supernatant were collected and frozen at -70°C for examination.

(2) Adipose tissue: a laparotomy was conducted after collecting serum at the end of each exposure to remove the perirenal fat pads for further study after cutting and freezing. This work selected adipose tissue for observation because of its significance in OSAHS (Obstructive Sleep Apnea-Hypopnea Syndrome) pathogenesis. Obesity is an independent risk factor for OSAS. Adipose tissue can further aggravate the condition of OSAHS patients.

(3) Tissue sample test: after the experiment, the blood of rats, which was chloral hydrate anesthetized and neck-cut, was drawn. After that, the hearts and renal arteries of all rats were collected and fixed in 10% formaldehyde solution for 24 hours for routine dehydration with gradient ethanol and then embedded in paraffin.

Establishment of Rat Model in Different Groups

The rats in the intermittent hypoxia group were placed in an alternating intermittent hypoxic chamber according to the Fletcher model production method; this chamber consists of a hypoxic chamber and two animal chambers (A, B). The hypoxic chamber was continuously filled with high-purity nitrogen gas and a constant concentration of oxygen (7.0±0.5%). The hypoxic chamber alternated between the animal chambers so that the two animal chambers were in a low-oxygen environment in the hypoxic chamber. Each intermittent hypoxic cycle was kept for 90 s. The low oxygen and normal oxygen concentration time were maintained for 30 s; nitrogen was added for 30 s and held for 15 s; then, oxygen was added for 30 s and held for 15 s; the gas flow was regulated to maintain the minimum oxygen concentration in each cycle (7.0 ± 0.5%). Next, the concentration gradually recovered to 21%. The rats were kept in intermittent hypoxia for 8 h each day, and they fasted without water during the experiment. A water absorbing agent and carbon dioxide adsorbent were placed in the chamber. The hypoxia experiment time was 9 am-5 pm each day and lasted 2-8 weeks for the different groups. Rats were fed with the same common diet. The groups were as follows: CIH 6 weeks group, CIH 8 weeks group, CIH 6 weeks + p38MAPK receptor inhibitor group, CIH 6 weeks + Tempol group, CIH 8 weeks + p38MAPK receptor inhibitor group and CIH 8 weeks + Tempol group.

(1) Normoxia control group: breathed normal air (also placed in animal chamber C), routine feeding for 4 weeks;

(2) CIH 2 weeks group: lived in the alternating intermittent hypoxic chamber for 2 weeks;

(3) CIH 4 weeks group: lived in the alternating intermittent hypoxic chamber for 4 weeks;

(4) CIH 6 weeks group: lived in the alternating intermittent hypoxic chamber for 6 weeks;

(5) CIH 6 weeks + p38MAPK receptor inhibitor group: lived in the alternating intermittent hypoxic chamber for 6 weeks with intraperitoneal injection of 200 nmol/mL p38MAPK

inhibitor SB203580 30 min before entering the hypoxic chamber with the dosage corresponding to the body weight (1 nmol /kg);

(6) CIH 6 weeks + Tempol group: lived in the alternating intermittent hypoxic chamber for 6 weeks, with an intraperitoneal injection of 10% Tempol 30 minutes before entering the hypoxic chamber with a dosage of 100 mg/kg (body weight);

(7) CIH 8 weeks group: lived in the alternating intermittent hypoxic chamber for 8 weeks;

(8) CIH 8 weeks + p38MAPK receptor inhibitor group: lived in the alternating intermittent hypoxic chamber for 8 weeks and received an intraperitoneal injection of p38MAPK inhibitor SB203580 30 minutes before entering the hypoxic chamber;

(9) CIH 8 weeks + Tempol group: lived in the alternating intermittent hypoxic chamber for 8 weeks and lived in the alternating intermittent hypoxic chamber for 4 weeks with an intraperitoneal injection of 10% Tempol 30 minutes before entering the hypoxic chamber, with the dosage of 100 mg/kg (body weight).

Experimental Test Method

(1) The changes in the oxidative stress index were evaluated in the rats of the different groups. The evaluated oxidative stress indexes were superoxide dismutase (SOD) and malondialdehyde (MDA). Serum SOD was evaluated with the hydroxylamine method, and serum MDA was measured with thiobarbituric acid chromatometry. The operations were conducted strictly following the kit specification.

(2) RT-PCR was used to detect the expressions of P-p38, P-JNK, P-ERK1/2 and TLR4 in rat adipose tissue. The rat adipose tissue in each model group was removed and ground into powder at 100 mg with liquid nitrogen. Total RNA was extracted using TRIzol method: the adipose tissue powder was transferred to 2 mL Eppendorf (EP) tube, added with 1 mL of TRIzol to stand for 10 min, and after with 200 μL of chloroform for mixing and shaking to stand for 5 min, centrifuged at 12000 r/min for 15 min at 4°C. The liquid supernatant was added with an equal volume of isopropanol to stand for 15 min and centrifuged at 12000 r/min for 4 min at 4°C. Next, the white precipitate at the bottom of the tube could be observed. The supernatant was discarded, and 1 mL of 75c/o ethanol was slowly added to the precipitate. The tube was mildly turned up and down to wash the tube wall and the precipitate at the bottom, and centrifuged at 12000 r/min for 5

min at 4°C. The ethanol was carefully removed to keep the precipitate. The centrifuge tube was dried at room temperature, and 25 μL DEPC water were added to dissolve the precipitate, which was then stored at -20°C for use. The total RNA purity was measured by UV spectrophotometer and the reverse RNA was reverse-transcribed using a reverse transcription kit: with 1 μg of total RNA as standard in a 20 μL reverse transcription reaction system at 37°C for 15 min and 85°C for 5 s, m RNA was reverse transcribed into cDNA. The target gene through PCR amplification: 4 μl of cDNA, 25 μl of Tap enzyme, 2 μL of each of the upstream and downstream primers were added into the Eppendorf (EP) tube. DEPC water was supplemented to 50 μL of the reaction system for PCR reaction. Amplification conditions: denaturation at 94°C for 45 s, annealing at 60°C for 90 s, extension at 72°C for 90 s, for 35 cycles. Finally extended at 72°C for 10 min. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference for PCR reaction. The primer sequence is as follows:
TLR4 (181 bp) Forward primer:
5′-CTGGGTTTCTGCTGTGGACA-3′
Reverse primer:
5′-AGGTTAGAAGCCTCGTGCTCC-3′
p38MAPK (111 bp)   Forward primer:
5′-GCACACTGATGACGAAATGACC-3′
Reverse primer:
5′-CCCACGGACCAAATATCCACT-3′
GAPDH (317 bp)   Forward primer:
5′-ATCACTGCCACTCAGAAG-3′
Reverse primer:
5′-AAGTCACAGGAGACAACC-3′

Next, 2% AGE on RT-PCR was performed. The electrophoresis results were collected using a computer gel scanning imaging system, and the grayscale ration of the target band and the internal reference GAPDH band were analyzed as the relative gene expression. Then, the semi quantitative analysis was conducted.

(3) Western-Blot was used to measure the expression levels of P-p38, P-JNK and P-ERK1/2 in rat adipose tissue. The rat adipose tissue in each model group was removed and grounded in liquid nitrogen. An appropriate amount of cell lysate with PMSF was collected for centrifugation at 12000 r/min for 15 min. Then the supernatant was obtained. The BCA Protein Concentration Assay Kit (Pierce, Rockford, IL, USA) was used for protein quantification. A 50 μg protein sample was taken for denaturation at 100°C for 5 min. Then, SDS-PAGE

(sodium dodecyl sulfate polyacrylamide gel electrophoresis) gel electrophoresis was performed. The sample was transferred to the PVDF (polyvinylidene difluoride) membrane (Millipore, Billerica, MA, USA) with TE22 protein transfer system. After blocking for 1 h with confining liquid (TBST + 5% skim milk powder), primary and secondary antibody incubation was performed for 2 h and 1 h, respectively. The sample reacted with an ECL (enhanced chemiluminescence) (Merck, Millipore, Billerica, MA, USA) developer after membrane cleaning and was exposed to X-ray in a dark room. Finally, the result was collected and analyzed using the JS-780 automatic digital gel imaging analysis system. The protein expression levels of P-p38, P-JNK and P-ERK1/2 protein were denoted as the ratio of the optical density value of the target protein band to the optical density value of the internal reference β-actin band.

(4) ELISA method was used to detect the expression of inflammatory cytokines in serum. The levels of serum IL-8, TNF-α, CRP and IL-6 were detected by ELISA method. All of the above operations were checked strictly according to the instructions of the kit.

Statistical Analysis

SPSS 18.0 software (SPSS Inc. Chicago, IL, USA) was used for data processing. All data were denoted as the mean ± standard deviation ($\overline{x}\pm s$), multiple sample comparisons were performed by one-way ANOVA, and pairwise comparison was conducted by using the t-test at the test standard α = 0.05. $p<0.05$ indicates the difference has statistical significance.

Results

Expression of Inflammatory Factors and Oxidative Stress Factors After Intermittent Hypoxia for 2 Weeks

The expression of inflammatory factors and oxidative stress factors after intermittent hypoxia for 2 weeks is shown in Figure 1. According to the results, with the increasing hypoxia intensity, SOD in the serum of CIH group was much lower than that in the control group, with a significant difference between CIH 2, 4, 6, and 8 weeks groups compared with the control group. With the increasing hypoxia intensity, the adiponectin level in serum of each CIH group was significantly lower than that in the normoxia control group. The levels of TNF-α, hsCRP, IL-8, MDA and IL-6 in serum were significantly greater than that in the normoxia control group, with a significant difference between CIH 2, 4, 6, and 8 weeks groups (Figure 1).
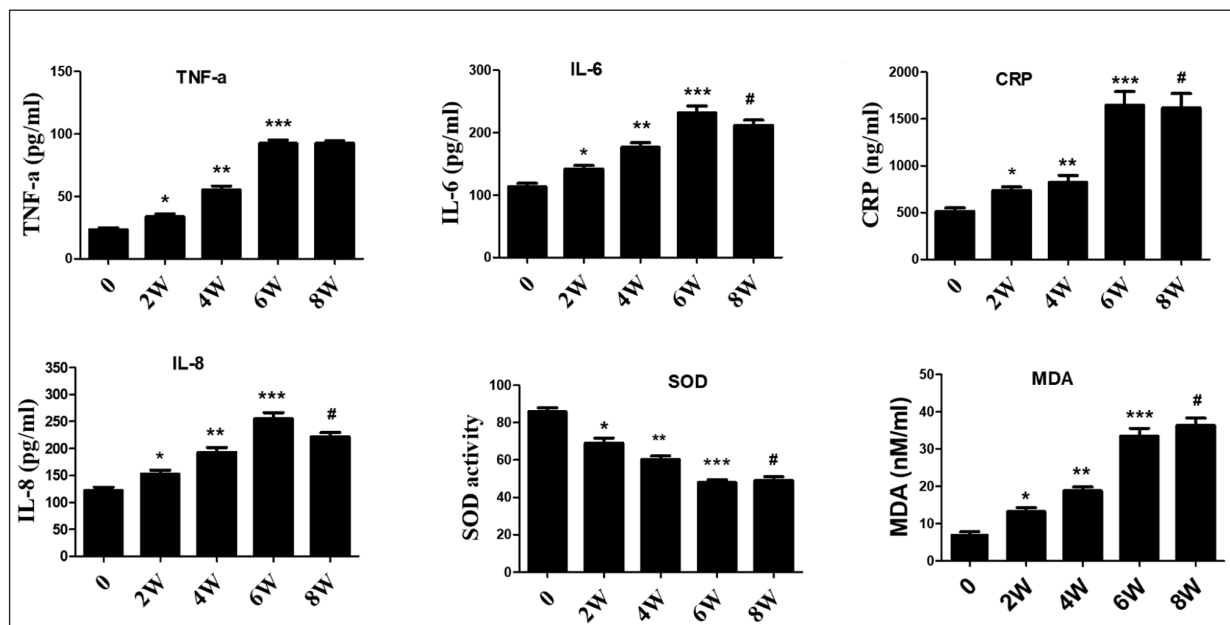

Figure 1. The expression trend of inflammatory factors and oxidative stress factors after 2 weeks of intermittent hypoxia. *$p<0.05$ vs. 0W. #$p<0.05$ *vs.* 6W.

Tempol Treatment

After treatment with the antioxidant Tempol in each group, the oxidative stress indicators improved. SOD increased significantly after Tempol treatment, and MDA decreased significantly; the levels were significantly different from those in the untreated groups (Figure 2). The inflammatory cytokines in serum improved. The levels of TNF-α, hsCRP, IL-8 and IL-6 in serum showed a significantly decreasing trend, with a significant difference from those in the untreated groups.

SB203580 Treatment

After treatment with the p38MAPK inhibitor SB203580, the SOD level significantly increased, and the level significantly decreased, indicating that the oxidative stress indicators were improved. After treatment p38MAPK inhibitor SB203580, the levels of TNF-α, hsCRP, IL-8 and IL-6 in serum gradually decreased and differed from those in the untreated groups (Figure 3).

Expression Changes of MAPK Pathway

Test results of MAPK pathway changes in rat adipose tissue indicated that P-p38, P-JNK, P-ERK1/2 and TLR4 proteins were expressed in rat adipose tissue. After intermittent hypoxia for 2, 4 or 6 weeks, the expression of P-p38 in the adipose tissue of rats significantly increased compared with that in the normal control group. The relative expression of P-p38 decreased in the intermittent hypoxia 6 weeks group and intermittent hypoxia 8 weeks group. There was an extremely small difference between the expression level of the P-JNK and P-ERK1/2 protein between groups. After 4 weeks of intermittent hypoxia with Tempol treatment, a decrease in p38MAPK protein expression was found, indicating that Tempol may inhibit the expression of the P-p38MAPK pathway. The TLR4 expression increased as hypoxia time increased, but Tempol did not inhibit TLR4 expression (Figure 4).

Discussion

OSAS refers to repeated airway collapse and obstruction during sleep, causing apnea and hypoventilation [13,14]. Normally OSAS is accompanied by snoring, sleep structure disorder and frequent decrease of oxyhemoglobin saturation[14].
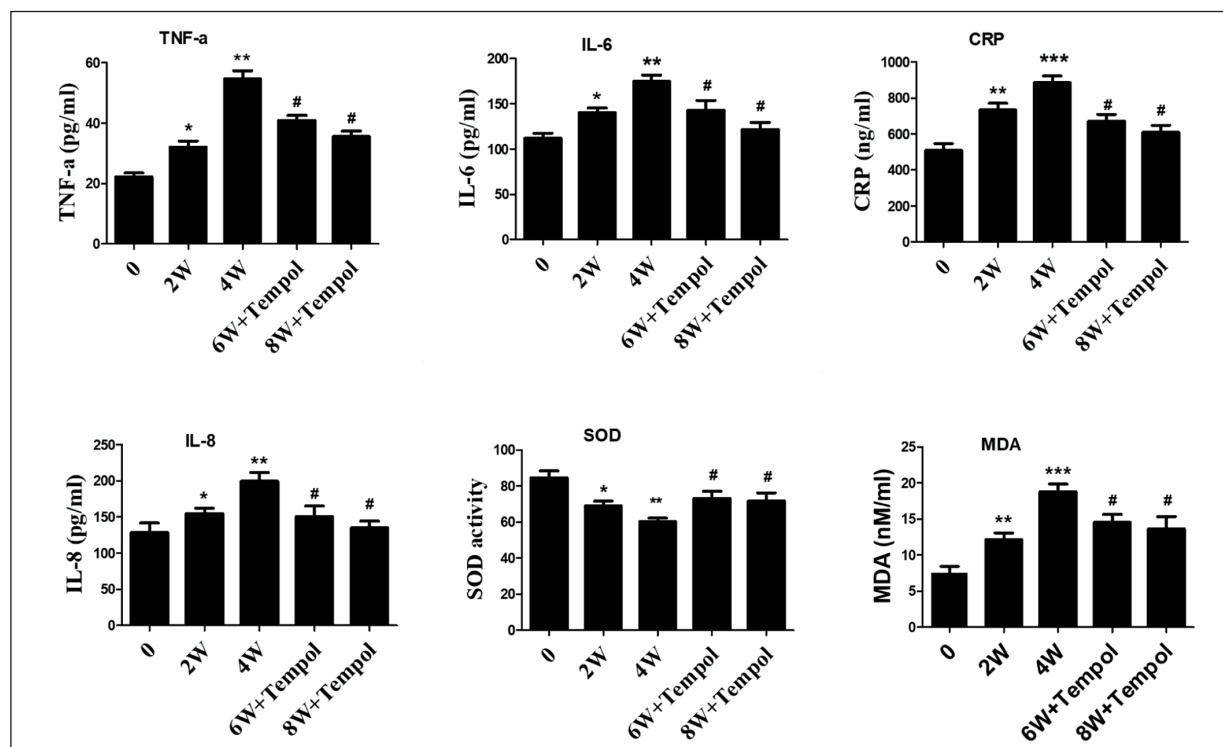

Figure 2. The expression of inflammatory and oxidative stress factors 2 weeks after intermittent hypoxia, and Tempol was used at the fourth week. *$p<0.05$ *vs.* 0W. #$p<0.05$ *vs.* 6W.
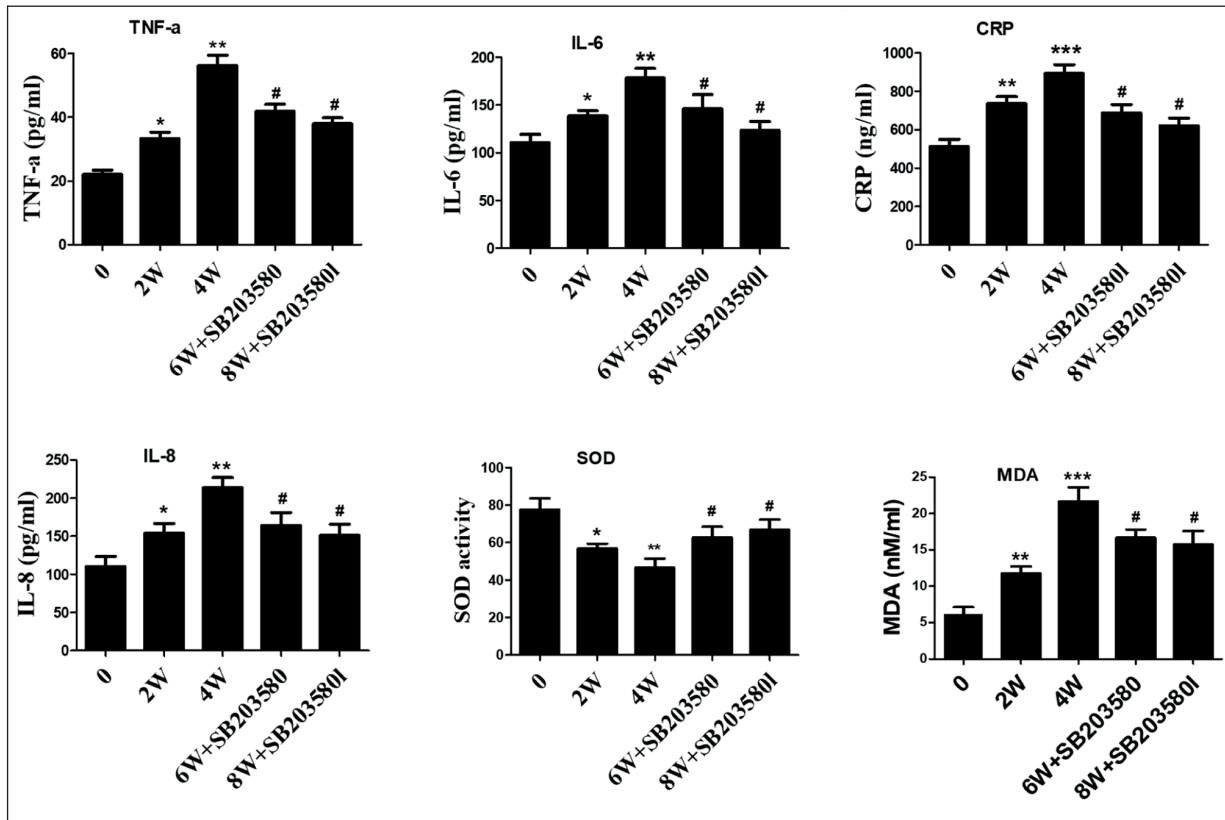
Figure 3. The expression of inflammatory and oxidative stress factors 2 weeks after intermittent hypoxia, and p-p38 inhibitor SB203580 was used at the fourth week. *$p<0.05$ *vs.* 0W. #$p<0.05$ *vs.* 6W.

OSAS intermittent hypoxia may activate inflammatory cells and increase the generation of CRP, IL-1, IL-8, IL-6 and TNF-alpha, etc., resulting in a systemic inflammatory reaction[15,16]. Independent of obesity, OSAS is a significant risk factor for insulin resistance. The CIH caused by repeated upper airway obstruction, which is the main pathological characteristics of OSAS and the most important factor triggering systemic inflammation, plays a key role in the occurrence and development of metabolic dysfunction. Currently, the precise mechanism by which intermittent hypoxia promotes metabolic disorders is unclear. The possible mechanisms include hypoxia itself, activation of the sympathetic nerves, systemic inflammation, activation of NF-kB and proinflammatory pathways, interleukin (IL-6), tumor necrosis factor (TNF), leptin, adiponectin and other insulin regulating hormone imbalance. Toll-like receptors have a role as the connection between nonspecific and specific immunity. TLR4, a member of the Toll-like receptor family is expressed in various cells, such as macrophages, fibroblasts, endothelial cells and cardiomyocytes.

TLR4 is also a pattern recognition receptor closely related to immune or inflammatory diseases. The known TLR4-mediated signal transduction pathways include My D88-dependence and My D88-independent pathways. The My D88-dependent pathway mediates the generation of MAPK and other signaling pathways[17]. p38MAPK, an important member of the MAPK family, is activated by external stimulation stress; it can promote the aggregation and activation of leukocytes and regulate transcription factor activity and cytokine synthesis, which are essentials in inflammatory reaction regulation[18]. TLR4 is an upstream molecule of p38MAPK, and there is an upstream and downstream connection between TLR4 and p38MAPK. TLR4 initiates the TLR4-mediated signaling pathway by developing a series of cascade reactions with relevant ligands[19]. Thus, TLR4 mediates p38MAPK activation, which is closely related to the expression of TNF-α, IL-6 and IL-1β inflammatory factors. Tempol, an outstanding free radical scavenger with intermediate function similar to those of superoxide dismutase can remove $O_2\cdot HO$, H_2O_2 and NO and various free
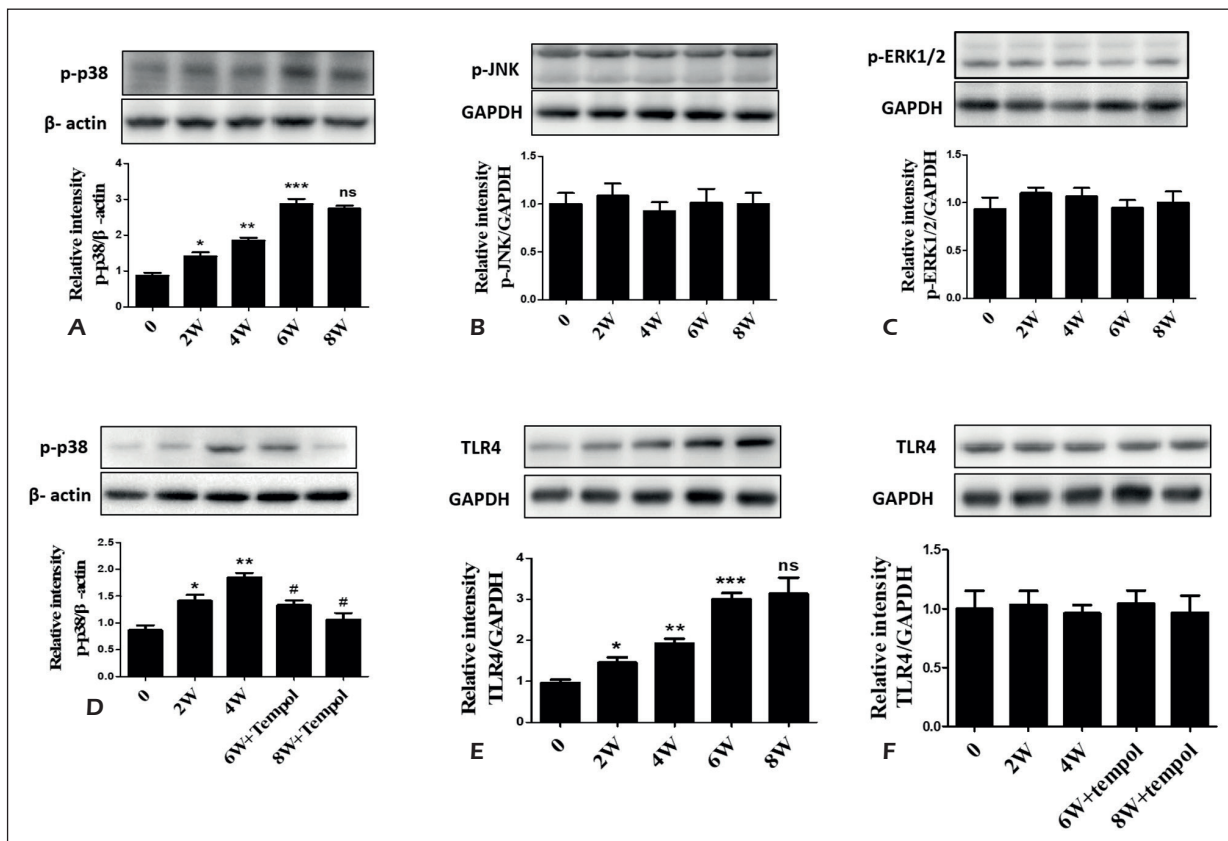
Figure 4. The changes of MAPK pathway expression. **A**, expression of p-p38 at different time points after intermittent hypoxia; **B**, expression of P-JNK at different time points after intermittent hypoxia; **C**, expression of P-ERK1/2 at different time points after intermittent hypoxia; **D**, diagram showed that Tempol could reduce the expression of P-p38, **E**, The TLR4 expression increased as hypoxia time increased; F, the Tempol did not inhibit the TLR4 expression. *$p<0.05$ *vs.* 0W. #$p<0.05$ *vs.* 6W.

radicals[20,21]. Moreover, Tempol has many biological activities such as anti-inflammation, radiation protection, ischemia-reperfusion injury protection and neuroprotection[22]. Additionally, Tempol is an efficient, low-toxic free radical scavenger, and it has been proved with a good protective effect on the nervous system[23]. The results showed that the SOD level in the serum of each CIH group decreased significantly when hypoxia intensity increased, and the difference among groups was significant. The adiponectin level in the serum of each CIH group was much lower than that in the normoxia control group if the hypoxia intensity increased, meanwhile, the levels of TNF-α, hsCRP, IL-8, and IL-6 were increased dramatically. After the Tempol and SB203580 treatment, the oxidative stress indicators improved such as SOD increased significantly and MDA decreased significantly. The inflammatory cytokine levels improved; the levels of TNF-α, hsCRP, IL-8 and IL-6 in serum showed an apparent decreasing trend, and the levels were significantly different from those in the untreated group. Additionally, the MAPK pathway proteins P-p38, P-JNK, P-ERK1/2 and TLR4 were found to be expressed in rat adipose tissue. The relative expression of P-p38 in the adipose tissue from the intermittent hypoxia 2 weeks group, intermittent hypoxia 4 weeks group and the intermittent hypoxia 6 weeks group increased significantly compared to the control group, but decreased in the intermittent hypoxic 6-weeks group and increased in the intermittent hypoxic 8 weeks group. The decreased expression of p38MAPK protein in intermittent hypoxic 4 weeks group with Tempol treatment, indicates that Tempol may inhibit the expression of the P-p38MAPK pathway. Since the TLR4 expression will increase with the extension of hypoxia time, the TLR4 expression after Tempol treatment without significant changes in this study demonstrates that Tempol didn't have an inhibitory effect on TLR4 expression.

Conclusions

We demonstrated that proteins of the TLR4-mediated p38MAPK signaling pathway are expressed in adipose tissue, and this expression increases with the duration of intermittent hypoxia. The expression of TLR4 and p38MAPK can regulate oxidative stress and inflammatory cytokine release. As indicated in animal experiments, the application of a p38MAPK inhibitor and an antioxidant free radical scavenger can improve the expression levels of oxidative stress markers and inflammatory cytokines. This study suggests new ideas and targeted drugs for treating OSAS patients, which will bring promising social and economic benefits.

Conflict of Interests

The authors declared no conflict of interest.

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