C1 inhibitor-mediated myocardial protection from chronic intermittent hypoxia-induced injury

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Abstract. The optimal treatment for chronic intermittent hypoxia (CIH)-induced cardiovascular injuries has yet to be determined. The aim of the current study was to explore the potential protective effect and mechanism of a C1 inhibitor in CIH in the myocardium. The present study used a rat model of CIH in which complement regulatory protein, known as C1 inhibitor (C1INH), was administered to the rats in the intervention groups. Cardiomyocyte apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. The expression of proteins associated with the apoptotic pathway, such as B-cell lymphoma 2 (Bcl-2), Bax and caspase-3 were detected by western blot analysis. The expression of complement C3 protein and RNA were also analyzed. C1INH was observed to improve the cardiac function in rats with CIH. Myocardial myeloperoxidase activity, a marker of neutrophil infiltration, was significantly decreased in the C1INH intervention group compared with the CIH control group, and cardiomyocyte apoptosis was significantly attenuated (P<0.05). Western blotting and reverse transcription-polymerase chain reaction analysis indicated that the protein expression levels of Bcl-2 were decreased and those of Bax were increased in the CIH group compared with the normal control group, but the protein expression levels of Bcl-2 were increased and those of Bax were decreased in the C1INH intervention group, as compared with the CIH group. Furthermore, the CIH-induced expression and synthesis of complement C3 in the myocardium were also reduced in the C1INH intervention group. C1INH, in addition to inhibiting complement activation and inflammation, preserved cardiac function in CIH-mediated myocardial cell injury through an anti-apoptotic mechanism.

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Introduction

Obstructive sleep apnea (OSA) is a commonly occurring disease, which is characterized by repeated upper airway collapse, increased breathing effort and decreased oxygen saturation. Exposure to chronic intermittent hypoxia (CIH), as observed in patients with OSA, has been found to be associated with a variety of cardiovascular (CV) diseases, including coronary heart disease, hypertension and chronic heart failure (1,2). On the basis of previous results reported in human and animal research, oxidative stress (3), inflammation (4) and sympathetic activation may be the key pathological processes involved in the pathogenesis of OSA-related CV diseases. However, the optimal treatment for CIH induced CV injuries has yet to be determined.

Complement regulatory protein, known as C1 inhibitor (C1INH), a serine protease inhibitor, is considered to be the only natural inhibitor of the complement pathway, and functions as a negative regulator of the complement system (5). The use of a highly specific and potent synthetic inhibitor of the activated C1 complement to block the classical complement pathway may be an effective strategy for the preservation of cells from hypoxic injury. Results of previous studies conducted by the present authors have demonstrated that the administration of C1INH protected cardiomyocytes against ischemia/reperfusion (I/R) injury-induced apoptosis (5,6). Therefore, the current study was conducted to investigate the hypothesis that C1INH may also exert protective effects against CIH-induced cardiomyocyte injury.

Materials and methods

Model of CIH in rats. Male Sprague-Dawley rats (n=60; weight, 200-250 g; age, 8-10 weeks) were purchased from Wuhan University (Wuhan, China). The rats were housed in an animal care center under a 12-h dark:light cycle (temperature, 20-26°C; humidity, 40-70%) and were allowed free access to standard chow and tap water. The rats were randomly divided into three large groups (n=20/group) as follows: i) Normal control (NC) group; ii) CIH group; and iii) CIH plus C1INH (CIH + C1INH) group. The rats were then further subdivided

into six smaller groups (n=10). The CIH rat model employed in the present study has been reported previously (7). Briefly, the rats were housed in a cage placed in a chamber of CIH. The chamber of CIH was a glass cube storehouse (width, 4 mm; length, 28x15x15 cm long), with a hole at one end (10 cm diameter) through which the animals were placed or removed, and two holes at the side (5 mm diameter) which was used to input low oxygen gas and air respectively. The concentration of the gas was regulated by an electromagnetic valve switch circuit which was controlled by a single chip microcomputer program, so that the input of low oxygen gas and air could be adjusted. The inspired oxygen fraction was altered from 21 to 4-5% every 2 min and sustained at the lower level for 20 sec. The intermittent hypoxia events were carried out for 4 or 8 weeks. Rats in the NC group were treated with 21% O_2 in a separate chamber. Rats in the CIH + C1INH group were also intravenously treated with C1INH (40 U/kg, 1 U 0.15 mg) per treatment, administered twice a week for 4 or 8 weeks (the effect of C1INH lasts for ≥3 days) (5). An injection of saline (0.5 ml per treatment) was administered to the NC and CIH groups. Data were collected after 4 or 8 weeks. The rats were anesthetized with ether inhalation, and the whole blood was collected prior to the measurement of cardiac function. The rats were then sacrificed by cervical dislocation. Tissue samples from the left ventricular were obtained. Some tissue samples were fixed in 10% paraformaldehyde, dehydrated, waxed, and embedded in paraffin, and others were homogenized.

Analyses of neutrophil infiltration, myocardial apoptosis, and cardiac C3 levels after CIH were performed, as described below. Homogenates were centrifuged at 36,000 x g at 4°C for 30 min. The supernatants were stored at -80°C.

Cardiac function. Echocardiographic examination was performed in conscious rats at 4 or 8 weeks after the initiation of CIH using a Sequoia 512 Echocardiographic System (Siemens Healthcare, Mountain View, CA, USA) equipped with an 8-MHz linear transducer. The anterior chest area of rats was shaved, and 2D images and M-mode tracings were obtained. The following parameters were measured: Left ventricular end-diastolic dimension (LVDd, in cm) and left ventricular end-systolic dimension (LVDs, in cm), and the results were used to calculate the left ventricular end-systolic volume [LVESV; $7.0/(2.4 + LVDs) \times LVDs^3$], left ventricular end-diastolic volume [LVEDV; $7.0/(2.4 + LVDd) \times LVDd^3$], left ventricular ejection fraction [LVEF (%); (LVEDV - LVESV)/LVEDV x 100] and left ventricular fractional shortening [LVFS (%); (LVDd - LVDs)/LVDd x 100].

Detection of myeloperoxidase (MPO) activity. As a marker of neutrophil infiltration, MPO activity in the myocardium and serum was determined using an MPO detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). TUNEL analysis was employed to evaluate myocardial apoptosis in the rats of each group. Briefly, myocardial tissue samples (5 μm) were rinsed in 1X phosphate-buffered saline (PBS; pH 7.4) and fixed for 30 min in 4% paraformaldehyde in 1X PBS (pH 7.4) at room temperature. Subsequent to rinsing with 1X PBS (pH 7.4), the cells were permeabilized in 0.1% Triton X-100 and 0.1% sodium citrate, then rinsed twice in PBS (pH 7.4). After washing with 1X PBS (pH 7.4), the cells were mounted in Gummi (Sinopharm Chemical Reagent, Co., Ltd.), an antifade agent, and visualized with an Olympus light microscope (Leica Microsystems GmbH, Wetzlar, Germany). Further characterization of apoptosis was achieved using a commercially available *in situ* cell death detection kit (Roche Diagnostics, Indianapolis, IN, USA) to detect DNA strand breaks using the TUNEL reagent, according to the manufacturer's protocol.

Western blot analysis. Left ventricular free wall tissue sample was obtained, and 100 mg myocardial tissue was placed into 1 ml lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mmol/l EDTA, 0.5 mmol/l PMSF, 4 g/ml peptide enzyme) prior to homogenization on ice. The sample was then centrifugated at 4°C (12,000 x g for 20 min), to obtain the supernatant, and the concentration of protein was detected by the Lowry method. The concentration of the total protein was adjusted to ~50 μg/15 μl. Protein samples (50 μg) were subjected to electrophoresis using a 10% sodium dodecyl sulfate-Tris-glycine polyacrylamide gel. The proteins were electrophoretically transferred to nitrocellulose membranes and then blocked with 5% fat-free milk in 1X PBS (pH 7.4), containing 0.05% Tween at 4°C overnight. The blocked membranes were incubated with anti-C3 (cat. no. sc-20137; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), B-cell lymphoma 2 (Bcl-2; cat. no. 2870-P; Cell Signaling Technology, Inc., Danvers, MA, USA), Bcl-2-associated X protein (Bax; cat. no. BS2538; Bioworld Technology, Inc., St. Louis Park, MN, USA), caspase-3 (cat. no. 19677-1-AP; Bioworld Technology, Inc.), β-actin (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China; cat. no. BM0627), or GAPDH (cat. no. AB-P-R001; Hangzhou Greensky Biological Tech. Co., Ltd., Hangzhou, China) antibodies (all diluted 1:2,000) in 5% fat-free milk for 2 h at room temperature, washed with 1X PBS (pH 7.4) for 20 min, and incubated with a 1:10,000 dilution of Immunopure anti-goat IgG (H+L) conjugated with horseradish peroxidase (cat. no. BA1054; Wuhan Boster Biological Technology, Ltd.) for 2 h at room temperature. Development of protein bands was performed using a SuperSignal Chemiluminescent Substrate kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The blots were quantified by grey value using analysis software (Bandscan 4.3; BioMarin Pharmaceuticals Inc., Novato, CA, USA).

Reverse transcription-polymerase chain reaction (RT-qPCR). The tissue samples were homogenized with liquid nitrogen, and total RNA was extracted in 1 ml TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration of the total RNA was detected by spectrophotometer. A First Chain Synthesis kit for cDNA (Fermentas) was used for reverse transcription. SYBR Green/Fluorescein qPCR Master Mix(2X) (Fermentas; Thermo Fisher Scientific, Inc., Pittsburg, PA, USA) and Ex *Taq*™ (Takara Bio, Inc., Otsu, Japan) were used

Table I. Echocardiographic data.

Variable	Normal	CIH (4 weeks)	CIH + C1INH (4 weeks)	CIH (8 weeks)	CIH + C1INH (8 weeks)
LVDd (cm)	0.556±0.050	0.580±0.048	0.563±0.476[a]	0.595±0.474	0.580±0.445[b]
LVDs (cm)	0.345±0.046	0.396±0.082	0.391±0.048[a]	0.427±0.064	0.394±0.044[b]
LVEDV (ml)	0.095±0.050	0.103±0.018	0.098±0.019[a]	0.144±0.031	0.133±0.025[b]
LVESV (ml)	0.504±0.036	0.537±0.046	0.527±0.048[a]	0.572±0.064	0.557±0.061[b]
LVEF (%)	82.04±3.11	77.49±3.50	78.88±2.53[a]	75.74±3.09	76.74±3.13[b]
LVFS (%)	41.14±2.39	38.98±2.36	39.78±2.49[a]	38.16±2.16	38.81±2.13[b]

Values represent means ± standard error of the mean (n=10/group). [a]P<0.05 vs. the CIH group at 4 weeks. [b]P<0.05 vs. CIH group at 8 weeks. CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

Table II. Effect of C1INH on MPO activity.

Group	MPO in myocardium (U/l wet weight)	MPO in blood (U/l wet weight)
Normal control	1.583±0.059	63.58±3.476
4 weeks		
CIH	2.580±0.128	90.69±4.048
CIH + C1INH	2.210±0.062[a]	88.76±4.019[a]
8 weeks		
CIH	4.014±0.11	161.22±3.00
CIH + C1INH	3.286±0.041[b]	131.98±4.53[b]

Values represent means ± standard error of the mean (n=10/group). [a]P<0.05 vs. the CIH group at 4 weeks. [b]P<0.05 vs. CIH group at 8 weeks. CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor; MPO, myeloperoxidase.

for RT-qPCR. RT-qPCR was employed to determine mRNA expression levels using the following primers: 5'-CTGCCT GTCCTTCAAAGTCCACC-3' and 5'-TCAGCATTCCAT CGTCCTTCTCC-3' for C3; and 5'-CACGATGGAGGGGCC GGACTCATC-3' and 5'-TAAAGACCTCTATGCCAACAC AGT-3' for β-actin. The reaction was carried out on an ABI Real-Time qPCR instrument. The reaction conditions were as follows: 94˚C for 4 min, 94˚C for 30 sec, 56˚C for 30 sec, 72˚C for 25 sec, and 30 cycles at 72˚C for 4 min. RT-PCR experiments were performed with 1 g of total RNA, followed by 40 cycles of PCR amplification. The expression levels were quantified using changes in the fluorescence signal through the analysis of the Cq value and standard curve; the starting template was quantitatively analyzed with RUO ViiA™ 7 software (Thermo Fisher Scientific, Inc.).

Statistical analysis. Data were expressed as the mean ± standard error of the mean, and analyzed using one-way analysis of variance followed by post-hoc analysis to compared the means. P<0.05 was considered to indicate a statistically significant difference.

Results

C1INH preserves cardiac function following CIH. C1INH administration significantly preserved cardiac function in rats in the CIH + C1INH group, compared with those in the untreated (CIH) group, as reflected by a significant increase in ejection fraction and fractional shortening, as well as decreased LVDd, LVDs, LVEDV and LVESV after C1INH treatment at 4 weeks (P<0.05) and 8 weeks (P<0.05) after intervention (Table I).

C1INH reduces inflammation in the myocardium following the induction of CIH. In the rat model of CIH, administration of C1INH was able to suppress the overactivated inflammatory response at 4 and 8 weeks, compared with that in the CIH group. This was revealed by the significant reduction of myocardial and serum MPO activity observed in rats in the C1INH intervention group at 4 and 8 weeks (P<0.05; Table II).

C1INH attenuates CIH-induced cardiomyocyte apoptosis. Cardiomyocyte apoptosis may be induced by numerous stimuli, including hypoxia. Administration of C1INH has been reported to be a potential cardioprotective strategy for hypoxia/reoxygenation-induced tissue injury (5). The presence of increased numbers of apoptotic cells in the myocardium was observable following 4 and 8 weeks of CIH (Fig. 1). C1INH, when administered at concentrations of 100-150 µg/ml (within the physiological concentration range in human plasma), markedly attenuated CIH-induced myocardial cellular apoptosis at both 4 and 8 weeks (Fig. 1). Furthermore, the protein expression levels of Bax induced by CIH were downregulated following treatment with C1INH, and Bcl-2 protein expression levels, which were reduced by CIH were upregulated by treatment with C1INH (Fig. 2). In addition, CIH-induced increased in myocardial caspase-3 protein expression were attenuated by treatment with C1INH (Fig. 2). The aforementioned data demonstrate that C1INH exerts an anti-apoptotic effect during CIH-induced myocardial injury.

C1INH inhibits CIH-induced cytoplasmic C3 synthesis in cardiac myocytes. The results of western blotting and RT-PCR analyses revealed that CIH at 4 and 8 weeks was associated
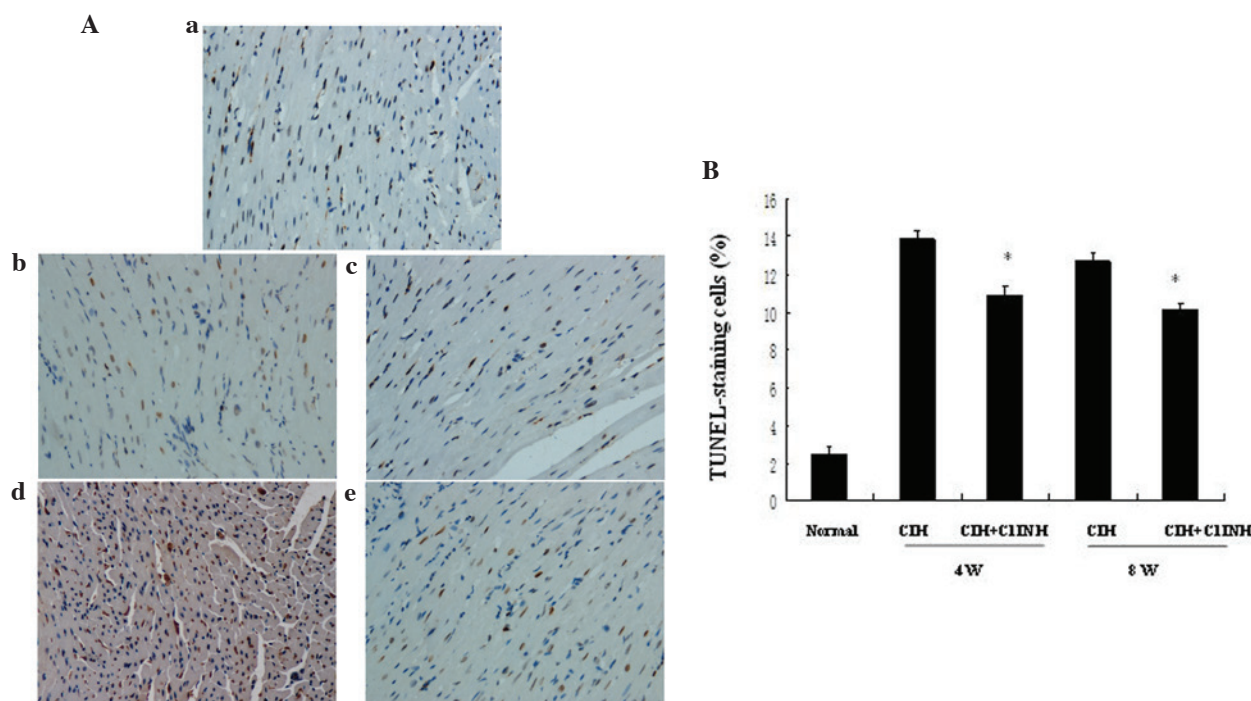
Figure 1. C1INH prevents myocardial cell apoptosis in rats undergoing CIH. Sectioned myocardial tissues from normal control rats and rats with CIH receiving 0.9% NaCl or 40 U/kg C1INH twice weekly for 4 or 8 weeks were analyzed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining. (A) Color photomicrographs of heart sections showed positive apoptotic nuclei in myocardial tissues at 4 and 8 weeks with *in situ* detection of DNA nick ends. Representative images from the (a) normal control, (b) CIH (4 weeks), (c) CIH + C1INH (4 weeks), (d) CIH (8 weeks) and (e) CIH + C1INH (4 weeks) groups are shown. Magnification, x400. (B) Quantitative analysis of TUNEL positively stained myocardial cells (%) was conducted based on the positive apoptotic nuclei staining in myocardial tissues at 4 and 8 weeks. Results (n=10) are presented as the mean ± standard error of the mean. CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor. *P<0.01, vs. the CIH group.

with increased cytoplasmic C3 protein and mRNA expression levels (Figs. 3 and 4, respectively). A single dose of C1INH (100 μg/ml) markedly reduced the C3 protein expression and cytoplasmic C3 mRNA expression induced by CIH. These results indicate that, in addition to its physiological function as the only inhibitor of the classical complement pathway proteases (C1r and C1s), C1INH may also exert a novel protective function against CIH-induced C3 synthesis in the myocardium.

Discussion

CIH has been suggested to negatively affect left ventricular function, and oxidative stress has been recognized as an important mediator for myocardial damage during this pathophysiological process (8). The major observation made within the current study was that C1INH supplementation during CIH preserved myocardial function, and attenuated myocardial apoptosis through the inhibition of complement activity.

Chronic intermittent hypoxia/reoxygenation is characteristic of CIH, and several studies support the notion that complement activation is involved in the pathogenesis of hypoxia/reoxygenation injury, and that complement inhibition reduces the extent of injury (5,8,9). In support of this hypothesis, studies have demonstrated that the localization/deposition of complement components (such as C1q, C3, C4 and C5) occurs in hypoxic myocardium (10-13). Furthermore, mice deficient in C3, C5 or C6 have less severe I/R-induced myocardial injury compared with those of the wild-type (11). In addition, depletion (with cobra venom factor) or inhibition (with C1INH

or soluble complement receptor 1) of complement activation attenuates cardiomyocyte injury during the hypoxia/reoxygenation process (12). C1INH has been demonstrated to block the classical complement pathway by binding to the activated C1 complex (C1q, C1s and C1r) (10), which results in dissociation of the complex. Finally, inactivated C1INH can be synthesized locally in inflamed tissue such as the I/R myocardium (13). The aforementioned data collectively suggest that activation of the complement system serves an important role in hypoxia/reoxygenation-induced myocardial injury.

Apoptotic cells are able to directly activate the classical complement pathway by binding C1 (14). Therefore, apoptosis contributes to hypoxia/reoxygenation-induced cell injury and organ dysfunction, and may serve as a target for the potential protective effects of acute phase proteins (15). Similarly, cellular apoptosis has been clearly documented in cultured neonatal rat cardiomyocytes experiencing hypoxia (16).

C1INH, a serine protease inhibitor, which has been used in the treatment of patients with paroxysmal nocturnal hemoglobinuria, has been evaluated for its potential clinical utility in studies of sepsis, I/R injury and capillary leak (17,18). It has been considered as a major inhibitor of complement system activation. C1INH is an acute phase protein that has a mean plasma level of ~250 mg/l and can be markedly upregulated up to 2.5-fold during the inflammation process (19). Previous data indicate that C1INH exerts an anti-apoptotic effect on vascular endothelial cell injury induced by gram-negative lipopolysaccharide (5). Therefore, inhibition of complement activation may attenuate CIH-induced myocardial apoptosis.
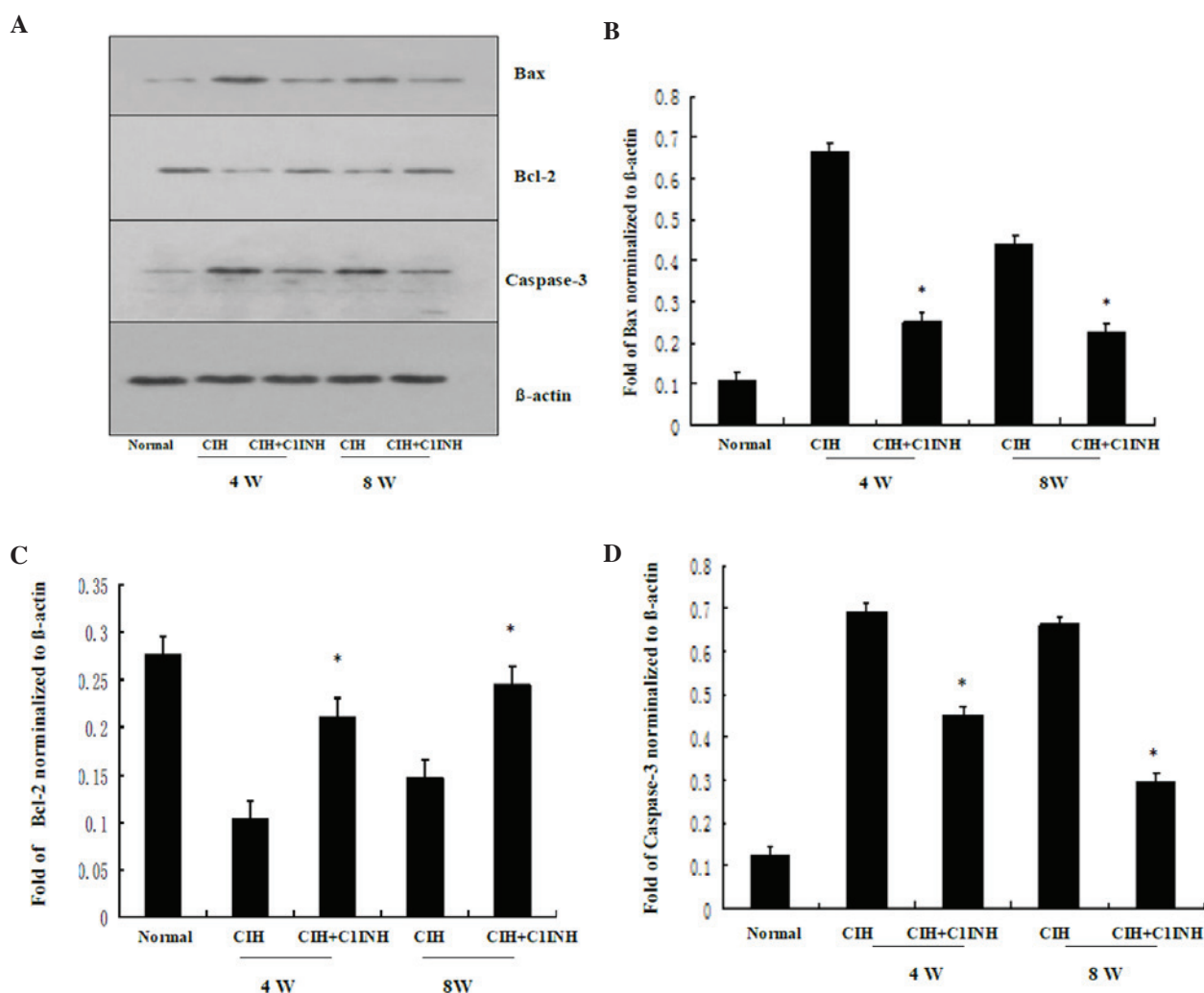
Figure 2. Effect of C1INH on Bcl-2, Bax and caspase-3 protein expression in the myocardial area of rats undergoing CIH. (A) Western blot analysis conducted to determine Bcl-2, Bax and caspase-3 expression levels in whole protein extracts from myocardial tissues in normal control rats and rats with CIH receiving 0.9% NaCl or 40 U/kg C1INH twice weekly for 4 or 8 weeks. Normoxic myocardial tissues from the normal control group and β-actin served as internal controls. A representative western blot from five independent experiments is shown. (B) Quantitative analysis of the effect of C1INH on Bax expression in myocardial tissues at 4 weeks and 8 weeks during CIH. (C) Quantitative analysis of the effect of C1INH on Bcl-2 expression in myocardial tissues at 4 weeks and 8 weeks during CIH. (D) Quantitative analysis of the effect of C1INH on caspase-3 expression in myocardial tissues at 4 weeks and 8 weeks during CIH. Results (n=10) are presented as the mean ± standard error of the mean. *P<0.01, vs. the CIH group. Bcl-2, B-cell lymphoma 2; CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor. .

In the present study, using an established CIH rat model, it was observed that C1INH was able to reduce myocardial apoptosis during CIH. Similarly, C1INH has been indicated to inhibit apoptosis in an H9c2 rat myocyte cell line *in vitro* (5), suggesting that a cardioprotective pathway may be initiated by C1INH. Furthermore, as C1INH is able to attenuate the apoptosis of H9c2 rat cardiomyocytes *in vitro*, it may be suggested that its anti-apoptotic effect is exerted through a mechanism that functions independently from the mediation of complement system activation (5). The mechanisms underlying the potential cardioprotective and anti-apoptotic effects of C1INH in myocardial cells have yet to be determined, and further investigation is warranted.

Although the activation of the complement system subsequent to hypoxia/reoxygenation injury has been attributed to various pathways, including the mannose-binding lectin pathway and the classical pathway for the heart, in addition to the alternative pathway for the kidney (20,21), complement C3 has been recognized as the most abundant complement protein in the circulation and is vital in the complement cascade. The classical, alternative and lectin pathways are activated via cleavage of the C3 molecule into various fragments that possess opsonic, chemotactic, anaphylotoxic and immunoregulatory properties. Typically, the liver produces the highest C3 levels under normal conditions; however, heart tissue undergoing hypoxia/reoxygenation produces substantially higher levels of C3 component compared with those in the liver (22). Local C3 synthesis may be an important pathological event during the complement-dependent processes, involving mediation of hypoxia/reoxygenation injury and immune-mediated tissue injury. Incremental increases in circulating C3a, and to a lesser extent C5a, are attenuated by C1INH treatment (23). The results of the present study also demonstrate that C1INH application is able to suppress the
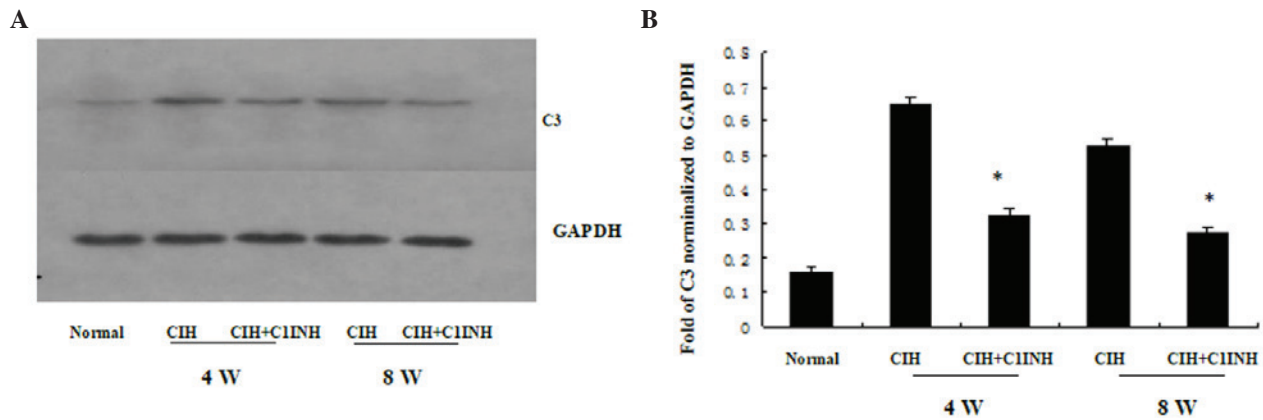
Figure 3. Effect of C1INH on C3 protein expression in the myocardium of rats undergoing CIH. Western blot analysis of C3 expression levels in whole protein extracts from the myocardial tissues in normal control rats and CIH rats receiving 0.9% NaCl or 40 U/kg C1INH twice weekly for 4 or 8 weeks. Normoxic myocardial tissues from the normal control group and β-actin served as internal controls. (A) Representative western blot from five independent experiments. (B) Quantitative analysis of the effects of C1INH on C3 expression levels in myocardium at 4 weeks and 8 weeks during CIH. Results (n=10) are presented as the mean ± standard error of the mean. *P<0.01, vs. the CIH group. CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase..
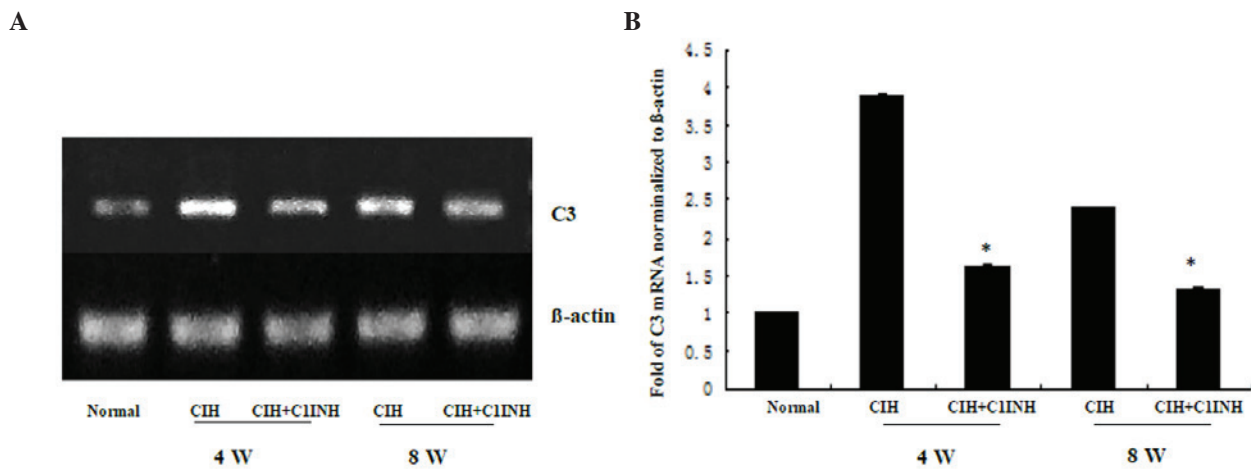

Figure 4. Effect of C1INH on C3 mRNA expression in the myocardium of rats undergoing CIH. RNA was isolated from the myocardial tissue of normal control rats and rats with CIH receiving 0.9% NaCl or 40 U/kg C1INH twice weekly for 4 or 8 weeks. C3 mRNA expression was analyzed by reverse transcription polymerase chain reaction. β-actin was the internal control. (A) A representative result is shown. (B) Quantitative analysis of the effects of C1INH on C3 mRNA in the myocardium at 4 weeks and 8 weeks during CIH. Results (n=10) are presented as means ± standard error of the mean. The ratio of the relative density of C3 mRNA in the myocardial tissue to that of the normal control was determined. *P<0.01, vs. the CIH group. CIH, chronic intermittent hypoxia; C1INH, C1 inhibitor.

upregulation of cytoplasmic C3 mRNA expression levels and protein synthesis in rat myocardium induced by CIH.

In addition, the inflammatory response in the myocardium involves the local production of chemotactic factors, neutrophil infiltration and activation, cytokine production and the expression of adhesion molecules. Thus, the ability of neutrophils to adhere to cardiac myocytes is enhanced, and the local activation of the complement system is induced (24). The results of the present study indicate that C1INH is required for the inhibition of neutrophil influx into the myocardium. Such effects on inflammation are most likely the result of direct prevention of early primary apoptosis or complement activation. Alternatively, direct anti-inflammatory effects of C1INH may also be involved. A reduction in neutrophil influx may explain the observed attenuation of apoptosis in rats treated with C1INH.

Collectively, the above results indicate that C1INH may exert its cardioprotective effects during CIH-induced myocardial cell injury and cardiac dysfunction through several different mechanisms, including the inhibition of complement activation induced by CIH, inhibition of proinflammatory events such as neutrophil accumulation, and the inhibition of locally synthesized C3. Thereby, application of C1INH may directly inhibit the activation of the complement pathway in hypoxic tissue. In addition, C1INH may also exert direct anti-apoptotic effects in CIH-mediated myocardial cell injury. These results collectively suggest that treatment with C1INH may provide a useful therapeutic approach for the treatment of patients with OSA.

Future animal studies are warranted to continue to enhance the present understanding of the pathogenesis of OSA-associated CV diseases, perhaps through the

investigation of knock-out and genetically engineered mice or by performing selective pharmacological interventions. This may enable identification of the fundamental molecular pathways underlying the association between OSA and CV diseases, and novel cardioprotective treatment options may emerge for the relatively large proportion of patients with OSA who are unable to tolerate continuous positive airway pressure therapy.

Acknowledgements

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