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Short-Term versus Long-Term Intermittent Hypobaric Hypoxia on Cardiac Fibrosis and Fas Death Receptor Dependent Apoptotic Pathway in Rat Hearts

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Abstract

It is unknown if short-term and long-term intermittent hypobaric hypoxic challenges both exert pro-apoptotic effects on Fas death receptor-dependent apoptotic pathway in rat hearts. Seventy-two Sprague-Dawley rats were randomly assigned into two groups. First, short-term intermittent hypobaric hypoxia (STIHH)-normobaric normoxia (n = 12), hypobaric hypoxia (380 mmHg, 12% O_2, 8 hrs/day) for 1 day (n = 12), and for 4 days (n = 12) and second, long-term intermittent hypobaric hypoxia (LTIHH)-normobaric normoxia (n = 12), hypobaric hypoxia for 1 week (n = 12) and 2 weeks (n = 12). After STIHH or LTIHH challenge, Fas receptor related pathway and histopathological analysis in the excised left ventricle was determined by Western blotting, RT-PCR, Hematoxylin-eosin staining, Masson trichrome staining and TUNEL assay. Fas death receptor and TNFα were significantly decreased after STIHH whereas Fas receptor, TNFα, FAS-associated death domain (FADD), and caspase 8 were increased after LTIHH. In addition, cardiomyocyte disarray and fibrosis were observed in 1 week LTIHH. Cardiac hypertrophy and more severe disarray, fibrosis and cardiac apoptotic activities were observed in 2 week LTIHH. STIHH exerts anti-apoptotic effects on hearts such as downregulation of TNFα and Fas receptor whereas LTIHH exerts pro-apoptotic effects such as upregulation of TNFα and Fas-mediated apoptotic pathways and lead to cardiac fibrosis and apoptosis. Our findings imply that short-term versus long-term intermittent hypobaric hypoxia exerted protective versus deleterious effects on hearts.

Key Words: cardiac fibrosis, cell apoptosis, high altitude, Fas pathway, time course

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Introduction

Apoptosis, a physiological program of cellular death, may contribute to many cardiac disorders, such as post-infarction myocardial apoptosis and heart failure (10, 13, 16). Cardiac apoptosis was found under various infections (20, 21), stressors (17) and in various modern diseases, such as obesity (19, 24), diabetes, and hypertension (15, 16). Fas (APO-1/CD95) is a tumor necrosis factor (TNF) receptor superfamily member (4, 10). Fas death receptor dependent (Type I) apoptotic pathway was thought to be one of the major pathway to trigger cardiac apoptosis (4, 10, 22). This pathway was initiated by death receptor agonists such as Fas ligand and TNFα (4, 6). Fas ligand binding followed by Fas-receptor oligomerization led to formation of a death-inducing signal complex starting with recruitment of the Fas-associated death domain (FADD) of the adaptor protein (4). Fas receptor oligomerization results in the activation of caspase 8 and causes the activation of apoptosis (4, 10, 22). After apoptosis, the collagens secreted by fibroblasts replace the space of cardiomyocyte losses or damages (25, 28). Hence, the cardiac fibrosis following myocardial apoptosis is recognized as a predictor of adverse outcomes in subjects with cardiomyopathies (14, 25). Therefore, the evaluation of apoptosis and/or fibrosis should be an important issue of predicting the development of hypoxia-induced cardiomyopathies.

Hypoxia-induced cardiomyocyte apoptosis or post-infarction myocardial apoptosis has been associated with Fas receptor dependent apoptotic pathway (3, 17, 29, 31, 36). In contrast, intermittent hypoxia has been shown to provide myocardial protection against ischemia/reperfusion-induced injury and attenuated ischemia/reperfusion-induced apoptosis (9). Our previous study presented opposite effects on Bcl2 family, which showed that anti-apoptotic Bcl-2 proteins were increased after 1 day and 4-day intermittent hypobaric hypoxia but decreased after 1-week and 2-week intermittent hypobaric hypoxia. In contrast, pro-apoptotic BNIP3 and Bad proteins were significantly decreased after 1-day and 4-day intermittent hypobaric hypoxia but increased after 1-week and 2-week intermittent hypobaric hypoxia (18). However, it is unclear if short-term versus long-term intermittent hypobaric hypoxic challenges exert similar pro-apoptotic effects or opposite effects on Fas death receptor dependent apoptotic pathway and cardiac fibrosis in rat hearts.

The purpose of the current study is to examine the effects of short-term versus long-term intermittent hypobaric hypoxia on Fas death receptor dependent apoptotic pathway. We hypothesized that short-term and long-term intermittent hypobaric hypoxic challenges may exert opposite effects on Fas death receptor dependent apoptotic pathway and cardiac fibrosis in rat hearts.

Materials and Methods

Animal Model

Male Sprague Dawley rats weighing 350~400 g at ate of 12 weeks were purchased from National Science Council Animal Center, Taiwan. All rats were housed three per cage. Ambient temperature was maintained at 25°C and the animals were kept on an artificial 12-h light-dark cycle. The light period began at 7:00 A.M. Rats were provided with standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*. All protocols were approved by the Institutional Animal Care and Use Committee of China Medical University, Taichung, Taiwan, and the principles of laboratory animal care (NIH publication) were followed.

Short-Term Versus Long-Term Hypobaric Hypoxia Exposures

A total of 72 rats were randomly divided into 2 groups, short-term intermittent hypobaric hypoxia (STIHH) versus long-term intermittent hypobaric hypoxia (LTIHH) (Fig. 1). The rats were placed in either a normobaric normoxic environment (760 mmHg, 21% O_2 and 79% N_2 for 24 h per day) or in hypobaric hypoxic chamber (380 mmHg, 12% O_2 and 88% N_2, for eight hours from 9:00-17:00 and 16 h normobaric normoxia per day). All rats were randomly subdivided into 6 groups, each as follows: [1] STIHH subgroup-normobaric normoxia for 1 or 4 days (n = 12), [2] STIHH subgroup-hypobaric hypoxia for 1 day (n = 12), [3] STIHH subgroup-hypobaric hypoxia for 4 days (n = 12); [4] LTIHH subgroup-normobaric normoxia for 1 or 2 weeks (n = 12), [5] LTIHH subgroup-hypobaric hypoxia for 1 week (n = 12), and [6] LTIHH subgroup-hypobaric hypoxia for 2 weeks (n = 12). (Fig. 1) After normobaric normoxic or hypobaric hypoxic exposure, the rats were weighed and decapitated. The eight hearts of animals per subgroup were excised and cleaned with ddH_2O. The left and right atrium and ventricle were separated and weighed. The ratios of the total heart weight and the left ventricular weight to body weight were weighed and calculated.

Tissue Extraction

Cardiac tissue extracts were obtained by homogenizing the left ventricle samples in a PBS buffer (0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a ratio of 100 mg tissue/0.5 ml PBS

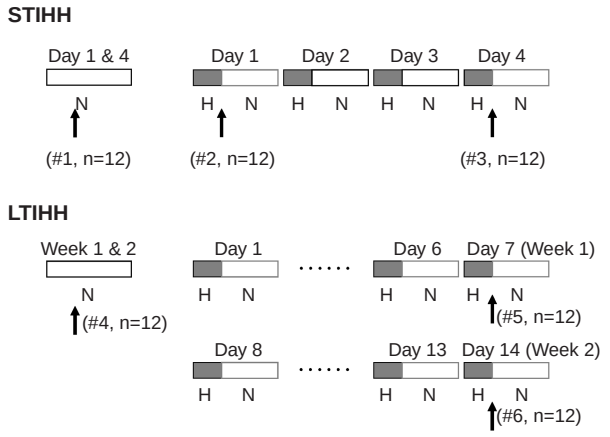

Fig. 1. Protocol diagram. Seven-two rats were randomly assigned into two groups, first, short-term intermittent hypobaric hypoxia (STIHH)- normbaric normoxia for 1 or 4-days (#1, n = 12), hypobaric hypoxia for 1-day (#2, n = 12), and 4-days (#3, n = 12) and second, long-term intermittent hypobaric hypoxia (LTIHH)- normbaric normoxia for 1 or 2-weeks (#4, normbaric normoxia, n = 12), 1 week (#5, n = 12), and 2 weeks (#6, n = 12). N = normobaric normoxia (about 760 mmHg, 21% O_2 and 79% N_2); H = hypobaric hypoxia (380 mmHg, 12% O_2 and 88% N_2, for 8 h per day); n, number of rats.

for 5 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000 rpm for 30 min. The supernatant was collected and stored at -70°C for further experiments.

Electrophoresis and Western Blot

The tissue extract samples were prepared as described by homogenizing with buffer. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done with 10% polyacrylamide gels. The samples were electrophoresed at 140 V for 3.5 h and equilibrated for 15 min in 25 mM Tris-HCl, pH 8.3, containing 192 mM glycine and 20% (v) methanol. Electrophoresed proteins were transferred to nitrocellulose membranes (Amersham, Hybond-C Extra Supported, 0.45 Micro) using a Bio-Rad Scientific Instruments Transphor Unit at 100 mA for 14 h. Nitrocellulose membranes were incubated at room temperature for 2 h in blocking buffer containing 100 mM Tris-HCl, pH7.5, 0.9% (w/v) NaCl, 0.1% (v/v) fetal bovine serum. Antibodies including Fas ligand, Fas, FADD, caspase 8 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and α-tubulin (Neo Markers, Fremont, CA, USA) were diluted 1:200 in antibody binding buffer containing 100 mM Tris-HCl, pH 7.5, 0.9% (w/v) NaCl, 0.1% (v/v) Tween-20 and 1% (v/v) fetal bovine serum. Incubations were performed at room temperature for 3.5 h. The immunoblots were washed three times in 50 ml blotting buffer for 10 min and then immersed in the second antibody solution containing alkaline phosphatase goat anti-rat IgG (Promega, Madison, WI, USA) for 1 h and diluted 1000-fold in binding buffer. The immunoblots were then washed in blotting buffer for 10 min three times. Color development was presented in a 20 ml mixture consisting of 7 mg nitro blue tetrazolium, 5 mg 5-bromo-4-chloro-3-indolyl- phosphate, 100 mM NaCl and 5 mM $MgCl_2$ in 100 mM Tris-HCl, pH 9.5.

RNA Extraction

Total RNA was extracted using the Ultraspec RNA Isolation System (Biotecx Laboratories, Inc.) according to the manufacturer's instructions. Each heart was thoroughly homogenized in 1 ml Ultraspec reagent/100 mg tissue using a Polytron homogenizer. The homogenates were washed twice with 70% ethanol by gentle vortexing. RNA precipitates were then collected by centrifugation at 12,000 g and dried under vacuum for 5-10 min before dissolving in 50 μl diethylpyrocarbonate-treated water, and then incubated at 55-60°C for 10-15 min.

RT-PCR

Total RNA was reversely transcribed and then amplified by the polymerase chain reaction using a Super Script Preamplification System for first strand cDNA Synthesis and Taq DNA polymerase (Life Technologies [GIBCO BRL], Rockville, MD, USA). RT-PCR products (45 μl) were separated on a 1.25% agarose gel (Life Technologies [GIBCO BRL]). Amplimers were synthesized by MdBio, Inc. based on cDNA sequences from Gene Bank. The rat GAPDH was used as an internal standard. The following rat primers were used: Rat TNFα forward primer-TCGAG TGACA AGCCC GTAG; Rat TNFα reverse primer-CAGAG CAATG ACTCC AAAGT AGAC; Rat GAPDH forward primer-GGGTG TGAAC CACGA GAAAT; Rat GAPDH reverse primer-CCACA GTCTT CTGAG TGGCA. Densitometric analysis of immunoblots and PCR was performed using AlphaImager 2200 digital imaging system (Digital Imaging System, San Leandro, CA, USA).

Hematoxylin-Eosin and Masson Trichrome Staining

After the hearts were removed from four rats per subgroup. They were soaked in formalin and covered with wax. Slides were prepared by first soaking for dehydration. They were passed through a series of graded alcohols (100%, 95% and 75%), 15 min of each. The slides were then dyed with hematoxylin and eosin or Masson trichrome. After gently rinsing with water, each slide was then soaked with 85% alcohol, 100%

Table 1. Effects of short-term (1 and 4 days) and long-term (1 and 2 weeks) intermittent hypobaric hypoxia on rat hearts

STIHH	Normoxia	Hypoxia: 1 day	Hypoxia: 4 days
Body weight (BW), g	390 ± 22	394 ± 10	370 ± 35
Whole heart weight (WHW), g	1.06 ± 1.08	1.1 ± 0.1	1.07 ± 0.12
Left ventricular weight (LVW), g	0.72 ± 0.07	0.73 ± 0.05	0.72 ± 0.08
WHW / BW ($\times 10^3$)	2.72 ± 0.22	2.79 ± 0.26	2.89 ± 0.41
LVW / BW ($\times 10^3$)	1.85 ± 0.27	1.85 ± 0.10	1.95 ± 0.28
LTIHH	Normoxia	Hypoxia: 1 week	Hypoxia: 2 weeks
Body weight (BW), g	416 ± 16	406 ± 27	404 ± 24*
Whole heart weight (WHW), g	1.13 ± 0.05	1.15 ± 0.07	1.25 ± 0.1*
Left ventricular weight (LVW), g	0.72 ± 0.03	0.75 ± 0.04	0.85 ± 0.01*
WHW / BW ($\times 10^3$)	2.72 ± 0.18	2.83 ± 0.30	3.1 ± 0.33*
LVW / BW ($\times 10^3$)	1.73 ± 0.11	1.84 ± 0.20	2.1 ± 0.25*

Values are means ± SEM (n = 8). STIHH, short-term intermittent hypobaric hypoxia; LTIHH, long-term intermittent hypobaric hypoxia. No significant differences between normobaric normoxia and hypobaric hypoxia 1 day or 4 days. *P < 0.05, significant differences between normobaric normoxia and hypobaric hypoxia for 1 week or 2 weeks.

alcohol I and II for 15 min each. At the end, they were soaked with Xylene I- Xylene II. Photomicrographs were obtained using Zeiss Axiophot microscopes. The data of cardiac fibrosis were computed and assessed according to stained blue areas with trichrome staining. For TUNEL assay, the sections were incubated with proteinase K, washed in phosphate-buffered saline, and incubated with permeabilisation solution, blocking buffer, then washed two times with PBS. The terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP for 60 min at 37°C using an apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA) was used for detection. TUNEL-positive nuclei (fragmented DNA) fluoresced bright green at 450-500 nm. The slides were observed for at least 5-6 separate fields × 2 slides × 3 LV regions (upper, middle, lower) excised from four rat hearts in each group.

Statistical Analysis

The data of whole heart weight index, protein levels, mRNA levels, and trichrome stained areas were compared among groups of animals in either short-term intermittent hypobaric hypoxia (STIHH)-three subgroups or long-term intermittent hypobaric hypoxia (LTIHH)-three subgroups using one-way analysis of variance (ANOVA) with pre-planned contrast comparison. In all cases, a difference at P < 0.05 was considered statistically significant.

Results

Heart Weight Index

The heart weights and ventricular weights were not significantly changed following 1-day and 4-days short-term intermittent hypobaric hypoxia (STIHH) (Table 1). The heart weights and ventricular weights were not significantly increased following 1-week long-term intermittent hypobaric hypoxia (LTIHH) whereas after 2-weeks LTIHH, significant cardiac hypertrophy was observed due to increased heart weight-to-body weight ratio (Table 1).

Components of Fas Receptor Dependent Apoptotic Pathway

To further understand another ligand associated with the Fas receptor dependent apoptotic pathway induced by short-term versus long-term hypobaric hypoxia, the gene expressions of the TNFα were measured by PCR. TNFα mRNA expression was significantly decreased following 1-day and 4-day STIHH (Fig. 2A), but in contrast was significantly increased following 1-week and 2-week LTIHH (Fig. 2B).

To further understand the Fas ligand and Fas receptor associated with the Fas receptor dependent apoptotic pathway induced by short-term versus long-term hypobaric hypoxia, the protein levels of the Fas ligand and Fas death receptor were measured by Western Blotting. Fas death receptor protein levels were significantly decreased following 1-day and 4-day STIHH (Fig. 3A), but in contrast were significantly increased following 1-week and 2-week LTIHH (Fig. 3B). However, Fas ligand protein levels were not changed following 1-day and 4-day STIHH or 1-week and 2-week LTIHH (Fig. 3).

To further understand the common domain, FADD associated with the Fas receptor dependent apoptotic pathway induced by short-term versus long-

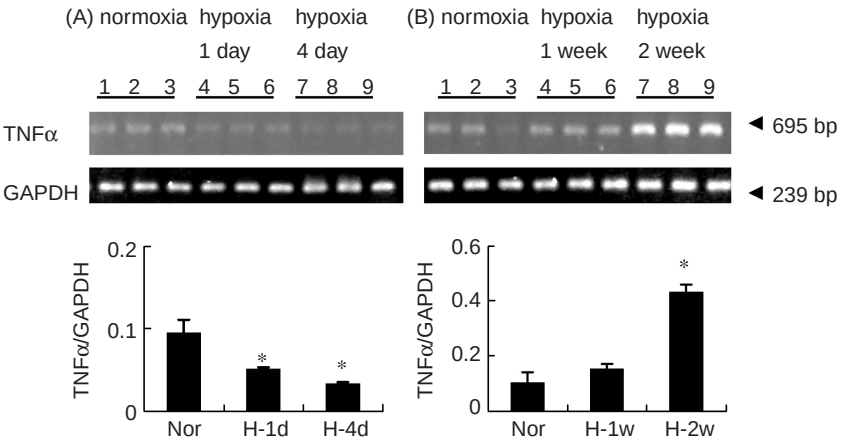

Fig. 2. The mRNA expressions of tumor necrosis factor alpha (TNFα) extracted from the left ventricles of rat hearts following two trials, (A) short-term intermittent hypobaric hypoxia *i.e*. normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-day group (H-1d, n = 3), and hypobaric hypoxia 4-day group (H-4d, n = 3); (B) long-term intermittent hypobaric hypoxia, *i.e*. normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-week group (H-1w, n = 3), and hypobaric hypoxia 2-week group (H-2w, n = 3), were measure by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Bars represent the relative quantification on the basis glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and indicate mean values ±SD (n = 9 in each group). **P* < 0.05, significant differences between normobaric normoxia group and hypobaric hypoxia group.

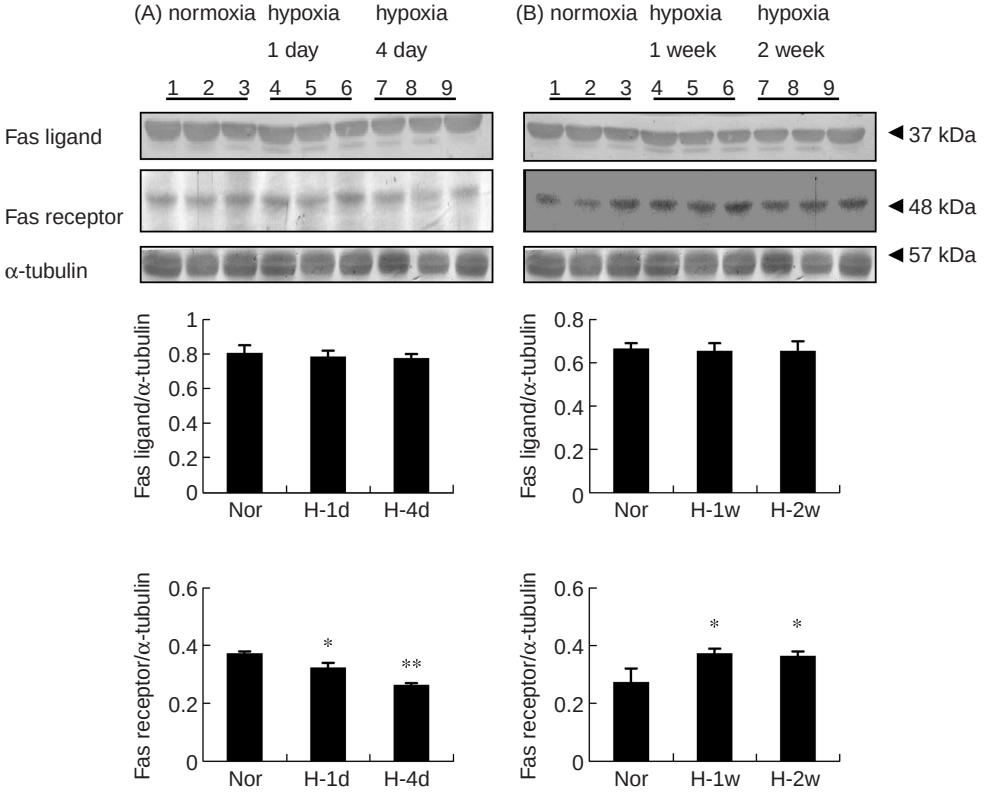

Fig. 3. The protein products of Fas ligand and Fas receptor extracted from the left ventricles of rat hearts following two trials, (A) short-term intermittent hypobaric hypoxia *i.e*. normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-day group (H-1d, n = 3), and hypobaric hypoxia 4-day group (H-4d, n = 3); (B) long-term intermittent hypobaric hypoxia, *i.e*. normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-week group (H-1w, n = 3), and hypobaric hypoxia 2-week group (H-2w, n = 3), were measure by Western Blotting analysis. Bars represent the relative quantification on the basis of α-tubulin and indicate mean values ±SD (n = 9 in each group). **P* < 0.05, ***P* < 0.01, significant differences between normobaric normoxia group and hypobaric hypoxia group.

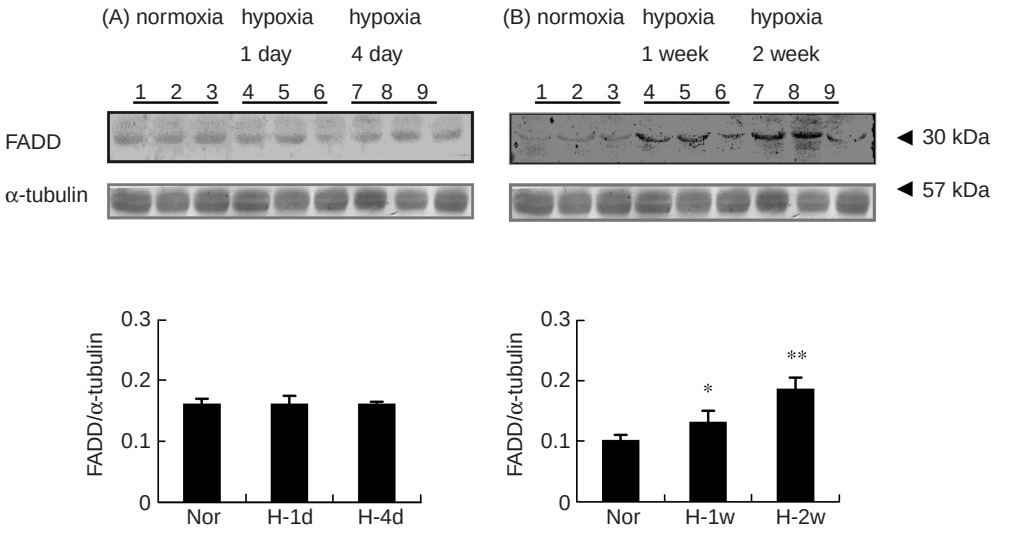

Fig. 4. The protein products of Fas-associated death domain (FADD) extracted from the left ventricles of rat hearts following two trials, (A) short-term intermittent hypobaric hypoxia *i.e.* normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-day group (H-1d, n = 3), and hypobaric hypoxia 4-day group (H-4d, n = 3); (B) long-term intermittent hypobaric hypoxia, *i.e.* normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-week group (H-1w, n = 3), and hypobaric hypoxia 2-week group (H-2w, n = 3), were measure by Western Blotting analysis. Bars represent the relative quantification on the basis of α-tubulin and indicate mean values ±SD (n= 9 in each group). * $P < 0.05$, ** $P < 0.01$, significant differences between normobaric normoxia group and hypobaric hypoxia group.

term hypobaric hypoxia, the FADD protein levels were measured by Western Blotting. FADD protein levels were not changed following 1-day and 4-day STIHH (Fig. 4A), but were significantly increased following 1-week and 2-week LTIHH (Fig. 4B). To further understand the Fas-mediated caspase activation induced by short-term versus long-term hypobaric hypoxia, the activated caspase-8 protein levels were measured by Western Blotting. Caspase-8 protein levels were not changed following 1-day and 4-day STIHH (Fig. 5A), but were significantly increased following 1-week and 2-week LTIHH (Fig. 5B).

Cardiomyopathic Changes

To understand the possible cardiomyopathic alteration after intermittent hypoxia, histopathological analysis of ventricular tissues with hematoxylin and eosin staining and Masson trichrome staining was performed. The ventricular myocardium under normbaric normoxia showed normal architecture with minimal interstitial fibrosis (Fig. 6, A and B). However, abnormal myocardial architecture and increased interstitial space were observed in rat hearts after 1-week LTIHH, and those observations become more obvious after 2-week LTIHH (Fig. 6A). Besides, hearts stained with Masson trichrome showed significant extensive fibrosis at minor levels, increased collagen deposition, and myofibril disarray in rats after 1 week and showed more obvious extensive fibrosis at moderate levels after 2-week LTIHH (Fig. 6B). No obvious differences of myocardial architecture, interstitial spaces, and fibrosis were observed following 1-day and 4-day STIHH (images not shown). No obvious differences of TUNEL-postive cardiac cells were observed following 1-day and 4-day STIHH whereas the increased TUNEL-postive cardiac cells were observed in rat hearts after 1-week LTIHH, and those observations become more obvious after 2-week LTIHH (Fig. 6C).

Discussion

Our main findings can be summarized as follows: Decreased Fas death receptor and TNFα were found in rat hearts following 1-day and 4-day STIHH, but in contrast Fas ligand, Fas death receptor, TNFα, FADD, activated-caspase 8, cardiac fibrosis, and cardiac apoptosis were all significantly increased following 1-week and 2-week LTIHH. Our major findings imply that short-term versus long-term intermittent hypobaric hypoxia exerted opposing effects on cardiac Fas receptor dependent apoptotic pathway on rat hearts.

Hypoxia will cause overall physiologic changes and responses, which involves in hemodynamics, pulmonary ventilation, circulation, hormonal regulation, neuromodulation and thermoregulation (17, 23, 32, 34). Therefore, cardiac changes in the current study may be caused by multiple factors challenged by hypoxia.

Fas death receptors respond to specific ligand

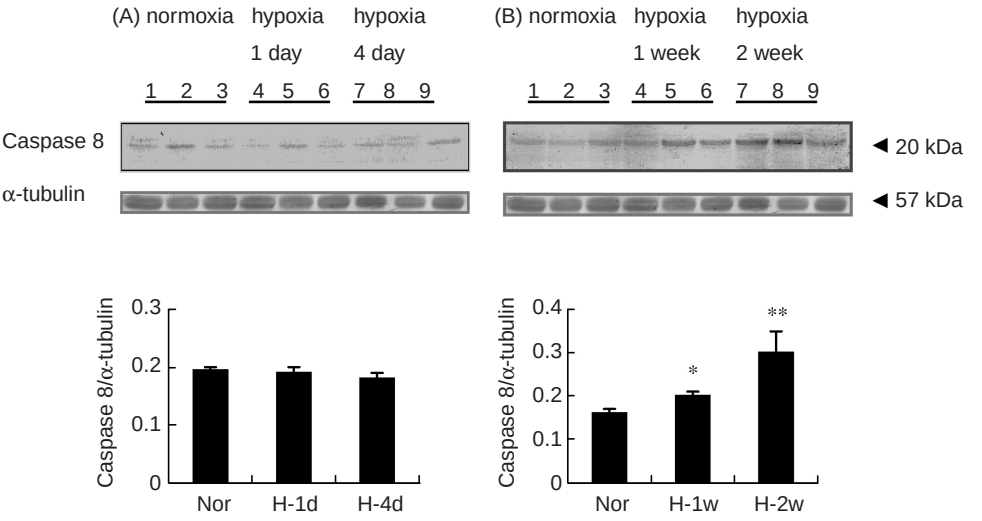

Fig. 5. The protein products of caspase 8 extracted from the left ventricles of rat hearts following two trials, (A) short-term intermittent hypobaric hypoxia *i.e.* normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-day group (H-1d, n = 3), and hypobaric hypoxia 4-day group (H-4d, n = 3); (B) long-term intermittent hypobaric hypoxia, *i.e.* normobaric normoxia group (Nor, n = 3), hypobaric hypoxia 1-week group (H-1w, n = 3), and hypobaric hypoxia 2-week group (H-2w, n = 3), were measure by Western Blotting analysis. Bars represent the relative quantification on the basis of α-tubulin and indicate mean values ±SD (n = 9 in each group). **P* < 0.05, ***P* < 0.01, significant differences between normobaric normoxia group and hypobaric hypoxia group.

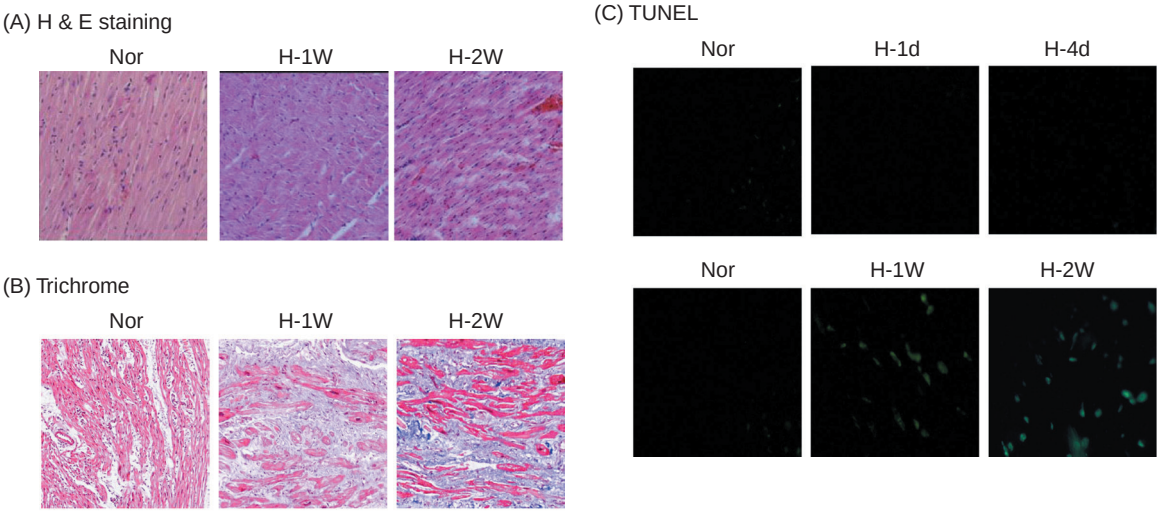

Fig. 6. Histopathological analysis of cardiac tissue sections stained with (A) hematoxylin and eosin as well as stained with (B) Masson trichrome were measured after normobaric normoxia group (Nor), long-term intermittent hypobaric hypoxia for 1 week (H-1w), and LTIHH for 2-weeks (H-2w). The images were magnified by 100 times. (C) Stained apoptotic cells of cardiac sections from left ventricles in rats were measured by stained with TUNEL assay with dark background (green spots) in STIHH (Nor, H-1d, H-4d) and LTIHH (Nor, H-1w, H-2w). The images of myocardial architecture were magnified by 100 times, and TUNEL assay was magnified by 400 times.

binding by Fas ligand or TNFα by recruiting FADD. FADD couples directly aggregates procaspase 8, leading to the cleavage and activation of caspase 8, which in turn induces cell apoptosis (4). In the current study, decreased Fas death receptor and TNFα after 1-day and 4-day STIHH imply that STIHH exert protective effects on Fas death receptor dependent apoptotic pathway and prevent from activation of caspase 8 and cardiac fibrosis. However, no changes of architecture and fibrosis were found in this short period. The current finding in the STIHH group further supports our previous findings “short-term

intermittent hypobaric hypoxia exerted protective effects on rat hearts", which showed that pro-apoptotic Bcl-2 family members, BNIP3 and Bad were significantly decreased whereas anti-apoptotic Bcl-2 family, Bcl2 were significantly increased after STIHH (18). Our current study is the first study to demonstrate downregulation of Fas death receptor and TNFα after STIHH but upregulation of Fas death receptor and TNFα gene expression after LTIHH.

In contrast, overall increases in Fas ligand, Fas death receptor, TNFα gene expressions, FADD, activated-caspase 8, and cardiac interstitial fibrosis after 1-week and 2-week LTIHH imply that LTIHH activated Fas death receptor dependent apoptotic pathway and led to cardiac fibrosis. The results in the LTIHH group are consistent with the notion that Fas-Fas ligand signaling pathway is activated in response to the different severity or various timing of hypoxia or ischemia/reperfusion (8, 12, 27, 31). The mRNA levels of Fas ligand, Fas death receptor, and activated caspase 8 were upregulated by simulated ischemia/reperfusion from cardiomyocytes in hypoxia (< 1% O_2) for 12 h and normoxia for 12 h (8). In an isolated Langendorff perfused rat heart model, ischemia and reperfusion induces the activation of caspase-8 in the heart (27). In cardiomyocytes with or without genetic manipulations, FADD/caspase-8 signaling was suggested as a primary role for cardiomyocyte apoptosis subjected to ischemia or serum deprivation (5).

The cardiac remodeling progresses with an increased level of collagenases immediately after myocardial damage (7). The collagens synthesized by the fibroblasts will invade and replace the apoptotic myocytes (2, 14, 25, 28). Accordingly, the accumulated collagens will further contribute to the development of ventricular fibrosis and heart failure (14). In our findings, abnormal myocardial architecture, increased interstitial space, increased cardiac fibrosis, and increased cardiac apoptosis following 1-week and 2-week LTIHH suggest that the development of cardiac apoptosis characterized by the distortion in myocardium architecture and cardiac fibrosis.

Our current findings in Fas receptor dependent apoptotic pathway and our previous findings in mitochondrial dependent apoptotic pathway (18) both strongly suggest that intermittent hypobaric hypoxia exerted protective or deleterious effect on rat hearts in a tightly time-course dependent. These findings may partially explain controversial effects of intermittent hypoxia or ischemia on cardiac damage or cardiac protection. After acute myocardial infarction (AMI), clinical trials had clearly shown the beneficial effects of early reperfusion within 12 h, and possibly up to 24 h (26), which can be explained by "open-artery hypothesis" (1). Our findings in STIHH might provide a partial explanation for the "open-artery hypothesis" which proposes that myocardial reperfusion, even if late for myocardial salvage, provides benefits and prevents adverse cardiac remodeling (1). On the other hand, our current findings in LTIHH might provide a partial explanation for "post-infarction myocardial apoptosis" (3) and "post-infarction cardiac fibrosis" (30, 37). Post-infarction myocardial apoptosis had been implicated as a cause of ongoing cell loss leading to cardiac failure and even the increased myocardial apoptosis occurred in the non-infracted remote myocardium (3). Since exercise training may promote cardiovascular health (33, 35) and STIHH can promote myocardial cell survival, for training applications, we may further propose that cardiopulmonary training under short-term challenges of intermittent hypobaric hypoxia may be beneficial for cardiac function, which might partially contribute to the improvement of "training high-living low" (11). Since LTIHH may aggravate myocardial cell death, for therapeutic and preventive application, we may further propose that there is a potential cardiac damage in people with "high mountain sickness" or in people ascending to high altitudes with a longer stay. Besides, it might be beneficial to block cardiac Fas signaling pathway when considering possible therapeutic agents to control the development of apoptosis and/or fibrosis-related cardiac diseases.

Acknowledgments

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