Effect of intermittent hypoxia on the cardiac HIF-1/VEGF pathway in experimental type 1 diabetes mellitus

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Abstract

Objective: High altitude and hypoxic preconditioning have cardioprotective effects by increasing coronary vascularity, reducing post-ischemic injury, and improving cardiac function. Our purpose was to examine if intermittent hypoxia treatment has any restoring effects related to the possible role of the HIF-1/VEGF pathway on diabetic cardiomyopathy.

Methods: Wistar Albino male rats (n=34) were divided into four groups: control (C), intermittent hypoxia (IH), diabetes mellitus (DM), and diabetes mellitus plus intermittent hypoxia (DM+IH). Following a streptozotocin (STZ) injection (50 mg/kg, i.p.), blood glucose levels of 250 mg/dL and above were considered as DM. IH and DM+IH groups were exposed to hypoxia 6 h/day for 42 days at a pressure corresponding to 3000 m altitude. Twenty-four hours after the IH protocol, hearts were excised. Hematoxylin and eosin-stained apical parts of the left ventricles were evaluated. Hypoxia inducible factor-1 (HIF-1), vascular endothelial growth factor 164 (VEGF164), and VEGF188 polymerase chain reaction products were run in agarose gel electrophoresis. Band density analysis of UV camera images was performed using Image J. The data were compared by one-way ANOVA, repeated measures two-way ANOVA, and the Kruskal-Wallis test.

Results: The percent weight change was lower in the DM group than in the controls (p=0.004). The tissue injury was the highest in the DM group and the least in the IH group. Diabetes decreased, whereas the IH treatment increased the vascularity. A decrease was observed in the VEGF188 mRNA levels in the DM+IH group compared with the C group, but there were no difference in HIF-1α and VEGF164 mRNA levels between the groups.

Conclusion: The IH treatment restored the diabetic effects on the heart by reducing tissue injury and increasing the capillarity without transcriptional changes in HIF-1/VEGF correspondingly. *(Anatol J Cardiol 2016; 16: 76-83)*

Keywords: angiogenesis, diabetic cardiomyopathy, intermittent hypoxia, HIF-1, VEGF

Introduction

Diabetes mellitus is a devastating metabolic disorder with multisystemic symptoms and complications. Its prevalence worldwide is continuously rising, 9.8% in men and 9.2% in women in 2008 (1). Cardiovascular complications such as coronary artery disease, cardiac autonomic neuropathy, and diabetic cardiomyopathy are the leading causes of diabetes-related morbidity and mortality (2, 3).

Diabetic cardiomyopathy is a condition with changes in the cardiac structure and function through hyperglycemia, dyslipidemia, and inflammation in the absence of hypertension and coronary artery disease (3). Hyperglycemia increases the levels of free fatty acids, reactive oxygen species, and growth factors and causes abnormalities in substrate supply and utilization, calcium homeostasis, lipid metabolism, and angiogenesis. Therefore, left ventricular hypertrophy, metabolic abnormalities, oxidative stress, apoptosis, extracellular matrix changes, fibrosis, intramyocardial microangiopathy, and impaired response to hypoxia are among the pathophysiological factors of diabetic cardiomyopathy (2, 4, 5).

Intermittent hypoxia has been known to protect the heart against lethal hypoxic insult by developing adaptive changes in the cardiac structure and function (6, 7). Hypoxia inducible factor-1 (HIF-1), a chief transcriptional regulator of hypoxic stimulus by controlling multiple responsive pathways including angiogenesis, plays an important role in intermittent hypoxia-induced cardioprotection (8-11). One of the HIF-1 transcriptional targets, vascular endothelial growth factor (VEGF), is crucial in the regulation of angiogenesis induced by myocardial ischemia/hypoxia and in the recovery of myocardial tissue from ischemic insult

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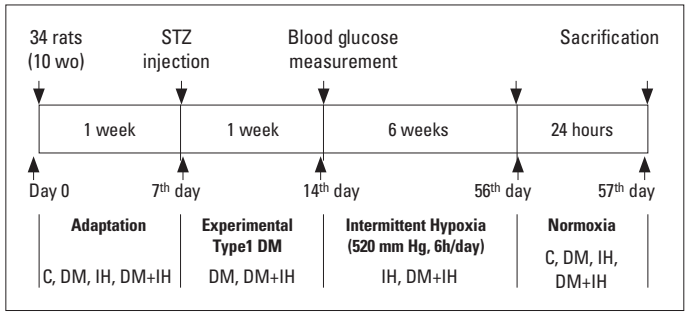

Figure 1. The summary description of experimental groups and their assigned protocols

C-control; DM-diabetes mellitus; DM+IH-diabetes mellitus+Intermittent hypoxia; IH-intermittent hypoxia; STZ-streptozotocin; WO-weeks old

(11, 12). Its expression is increased in cardiac myocytes and arteriolar smooth muscle cells following myocardial infarction in nondiabetic patients (13). HIF-1alpha mRNA is also up-regulated in patients with acute ischemia or early infarction, followed by VEGF increase in patients with infarction (14). Furthermore, acute and intermittent hypoxia has been shown to increase cardiac HIF-1 and VEGF levels in animal experiments (15-17). On the other hand, in patients with diabetes, VEGF is decreased and angiogenesis is impaired by hyperglycemia. These changes contribute to the pathophysiology of diabetic cardiomyopathy (18, 19).

Intermittent hypoxia may have potential therapeutic effects on cardiac dysfunction in diabetes by improving some of the causative factors. In our experimental design, the first step to investigate this speculation is to determine if intermittent hypoxia has any impact on cardiac changes occurring by the diabetic stimulus. Therefore, in the present study, we aimed to investigate the effects of intermittent hypoxia on cardiac tissue injury, changes in coronary angiogenesis, and the HIF-1/VEGF pathway in rats with type 1 diabetes with possible cardiomyopathy. We used a streptozotocin (STZ)-induced diabetes model. The use of STZ in animal models to deplete beta cells in the pancreatic islets is a well-established method for the imitation of type 1 diabetes, which in fact, results from the permanent destruction of the beta cells mostly by autoimmunity or environmental factors such as diet and viruses (20). Furthermore, diabetic cardiomyopathy, evident by contractile dysfunction, develops after the 5th week of diabetes in STZ-induced diabetic rats (21).

Methods

Animals

Adult male Wistar Albino rats (10 weeks old, weighing 217.9±18.3 g) were obtained from Ankara University School of Medicine. The animals were housed in Animal Research Laboratory of School of Medicine for a week before the experiments began. Water and standard rat food were provided ad libitum and 12-h light/dark cycles were provided using automated lighting system. All animal experiments were performed under the guidelines on human use and care of laboratory animals for biomedical research published by National Institutes of Health (8th ed., revised 2011) and conformed with the Declaration of Helsinki. The Ethics Committee of Ankara University approved the experimental protocol (No: 2012-11-79, Date: 05.23.2012).

Experimental groups and protocols

Thirty-four weight- and age-matched male rats were randomly divided into four groups: control (C, n=7), intermittent hypoxia (IH, n=9), diabetes mellitus (DM, n=8), and diabetes mellitus + intermittent hypoxia (DM+IH, n=10). Group C had no intervention, but the animals were kept under the same environment during the other experiments and their tissues were extracted at the same time as that of the other groups. Group IH was exposed to hypoxia 6 h/day for 6 weeks in a hypobaric hypoxia chamber (APCU-01, Betlehem). The pressure was kept at 69.3 kPa (520 mm Hg), which corresponds to an altitude of 3000 m (22). This level of hypoxia is accepted as high altitude, which already has potential pathophysiological effects (23). All hypoxia experiments were performed between 9:00 a.m. and 3:00 p.m. The tissues were extracted 24 h after the last hypoxia session (57th day of the experiment). The animals of the DM and DM+IH groups were injected with a single dose of STZ (50 mg/kg, i.p.) in freshly prepared citrate buffer (0.1 M, pH 4.5) (24, 25). One week later, the blood glucose levels from the tail venous blood of the animals were measured using a glucose meter (Optium Xceed, Abbott). The animals were considered diabetic if the blood glucose level was ≥250 mg/dL. Following this confirmation, the group DM was kept under normoxic conditions with group C, whereas group DM+IH was exposed to hypoxia with the group IH. The weights and blood glucose levels of the rats were measured weekly. The experimental groups and protocols are summarized in Figure 1.

Tissue extraction and molecular studies

Following the last hypoxia treatment, rats were anesthetized with thiopental sodium injection (50 mg/kg, i.p.). When no response to the pain stimulus by toe squeezing was observed, the thorax was opened and the heart tissue was extracted. The left and right ventricles were separated and the excess blood was washed with saline. The apical part of the left ventricle was stored in 10% formalin for histological evaluation. The remaining tissue samples were immediately shocked with liquid nitrogen and stored at -80°C for polymerase chain reaction (PCR) analysis.

Total RNA isolation

Total RNA samples of left ventricles were isolated using a commercial isolation kit for fibrous tissues (Fibrous Tissue Mini Kit K74704, Qiagen). Briefly, mechanochemical tissue homogenization was performed in liquid nitrogen and several buffers using a tissue homogenizer system (Glas-Col, 099C-K5424). Following the proteinase incubation of the homogenate, total RNA from the samples were eluted in spin columns with several centrifuging and washing steps. Subsequently, the concentration and quality of total RNA samples were measured at 230 nm, 260 nm, and 280 nm (NanoDrop, ND-1000). The ratios of 260/280

and 260/230 were considered for the purity and quality of RNA and the extractions were repeated until the 1.8-2 ratio was achieved. All total RNA samples were run on 1% agarose gels to check their integrity. The observance of intact 28S and 18S RNA bands were required to continue further experiments with this sample (Fig. 2).

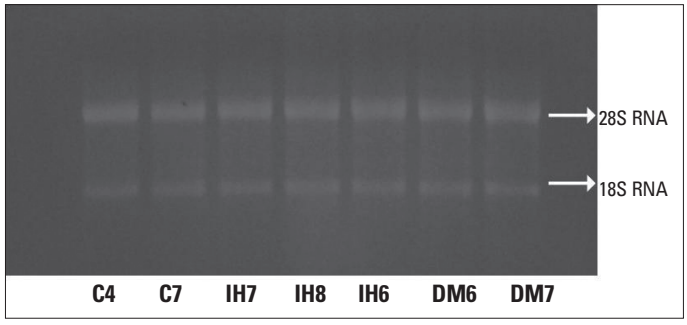

Figure 2. The intact 28S and 18S RNA bands of total RNA samples
C-control; DM-diabetes mellitus; IH-intermittent hypoxia

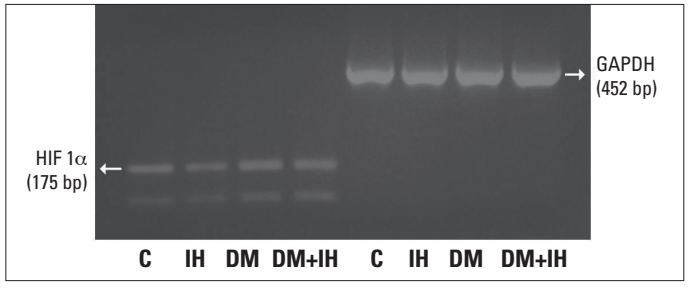

Figure 3. Samples of 2% agarose gel showing HIF-1α mRNA expressions in the left ventricle of the hearts from the experimental groups
C-control; DM-diabetes mellitus; IH-intermittent hypoxia

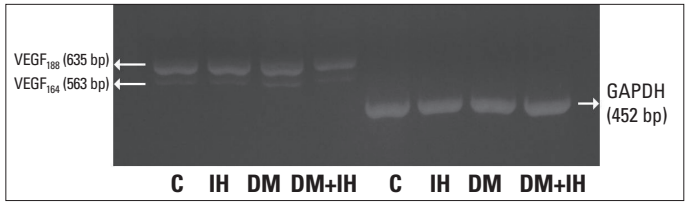

Figure 4. Samples of 2% agarose gel showing VEGF188 and VEGF164 mRNA expressions in the left ventricle of the hearts
C-control; DM-diabetes mellitus; DM+IH-diabetes mellitus+Intermittent hypoxia; IH-intermittent hypoxia

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA (2 µg) per sample was converted to total cDNA by reverse transcriptase using a commercially available reverse transcription kit (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas, Life Sciences, European Union). To obtain specific mRNAs, total cDNAs were amplified by PCR using rat HIF-1α, VEGF, and GAPDH (house-keeping gene) specific primers (26, 27). The gene regions corresponding to these primers were double-checked from NCBI, Nucleotide Database (http://www.ncbi.nlm.nih.gov/nucleotide) and Ensemble Genome Browser Database (http://useast.ensembl.org). The base-pair counts of targeted PCR products were calculated and optimal PCR conditions were adjusted according to the base sequences (Table 1).

Agarose gel electrophoresis and mRNA analysis

PCR products (15 µL) along with the DNA marker were run on 2% agarose gel with ethidium bromide at 100 volts for 1 h. The mRNA bands stained by ethidium bromide in the gel were visualized under UV light by a digital camera (Cleaver Scientific, DIHD) and the pictures were transferred to a computer. To confirm if the obtained cDNA bands were corresponding to the specific genes, the localizations of the sample's bands were compared to the bands of a DNA marker [PhiX174 DNA/BsuRI (HaeIII) Marker, 9] with known standard base pairs. The band density was measured using a software program (Image J 1.38X, Wayne Rasband, NIH, USA) (28). The relative contents of the VEGF and HIF-1α mRNAs were calculated as a proportion of the density of GAPDH mRNA for each sample. All measurements were triplicated (Fig. 3, 4).

Histological studies

The apical part of the left ventricle was buffered in 10% formalin for 3 to 7 days. Following the routine fixing, washing, and dehydrating steps, the tissue was embedded into paraffin blocks and 5-µm slices were obtained using the Leica RM 2125RT sliding microtome. The tissue slices were stained with hematoxylin and eosin and visualized under a light microscope (Carl Zeiss Axioskop, Göttingen, Germany). The myocardial tissue integrity and vascularization were examined. Microvascular density (MVD) was measured in 20× cross-sectional fields of 660×880

Table 1. PCR conditions and base sequences of the primers

Target DNA	Primer sequences (5′-3′)	PCR Program (/30 cycle/)	Target base #
HIF-1	f: AAG TCT AGG GAT GCA GCA r: CAAGATCACCAGCATCTAG	94°C(3′)/94°C(30′′)-54°C(30′′)-72°C(1′)/72°C(5′)	175 bp
$VEGF_{188}$	f: CTGCTCTCTTGGGTGCACTG r: CACCGCCTTGGCTTGTCACAT	94°C(3′)/94°C(30′′)-60°C(30′′)-72°C(1′)/72°C(5′)	635 bp
$VEGF_{164}$	f: CTGCTCTCTTGGGTGCACTG r: CACCGCCTTGGCTTGTCACAT	94°C(3′)/94°C(30′′)-60°C(30′′)-72°C(1′)/72°C(5′)	563 bp
GAPDH	f: ACCACAGTCCATGCCATCAC r: TCCACCACCCTGTTGCTGTA	94°C(3′)/94°C(30′′)-60°C(30′′)-72°C(1′)/72°C(5′)	452 bp

GAPDH - glyceraldehyde 3-phosphate dehyrogenase; HIF-1 - hypoxia inducible factor-1; PCR - polymerase chain reaction; $VEGF_{164}$ - vascular endothelial growth factor 164; $VEGF_{188}$ - vascular endothelial growth factor 188

Table 2. The blood glucose levels (mg/dL) from baseline, 15th day, and 50th day of the experimental groups (mean±SD)

Groups	Experiment (1st day)	Experiment (15th day) (1st day of hypoxia)	Experiment (50th day) (36th day of hypoxia)
C	88.2±21.3	81.6±13.7	81.2±4.8
IH	92.2±13.5	95.6±23.3	82.3±6.7
DM	96.6±17.3	410.5±45.1	379.8±86.3
DM+IH	103.4±17.6	366.6±53.5	328.3±71.8

C - control; DM - diabetes mellitus; DM+ICH - diabetes mellitus + intermittent hypoxia; ICH - intermittent hypoxia. According to the results of repeated measures two-way ANOVA, blood sugar group interaction was significant (p<0.001). Paired comparisons showed that DM vs. C and IH (p<0.001), and DM + IH vs. C and IH (p<0.001) were significant

Table 3. The weight measures (g) of the animals at baseline and sacrification day (mean±SD) and the percentage changes

Groups	Baseline	57th day	% change
C	209.7±11.0	298.4±18.4	42.3±5.3
IH	210.1±16.3	327.0±49.1	55.0±13.1
DM	232.3±19.4	259.0±22.3	11.6±5.9*
DM+IH	219.2±17.8	279.0±27.5	27.7±12.4#,×

C - control; DM - diabetes mellitus; DM+ICH - diabetes mellitus + intermittent hypoxia ICH - intermittent hypoxia. *p<0.001 DM vs. C and ICH, #p<0.05 DM+IH vs. C and DM, ×p<0.001 DM+IH vs. IH

μm, randomly selected from the intensely vascularized area and the results were expressed as an average of three fields.

Statistical analysis

The statistical analyses were performed using SPSS 15.0 program (SPSS Inc. and Lead Tech. Inc., Chicago, USA). The values were presented as mean ± SD. The statistical evaluation was accepted as significant when the p-value for each test is ≤0.05. The relative mRNA band densities of each gene were compared among the four experimental groups using one-way ANOVA. Blood glucose levels were evaluated using repeated measures two-way ANOVA. The percentages of the weight change before and after the experiment among the four groups were also compared using one-way ANOVA. The following calculation was applied to determine percentage change: % change=[(after-before)/before]×100. MVD results were compared by Kruskal-Wallis test. The comparison between each two groups was made by Tukey's post-hoc test.

Results

Blood glucose levels

The blood glucose levels of 3 days (1st, 15th, and 50th days) obtained from the weekly measures during the experiments are presented in Table 2. According to the results of repeated measures two-way ANOVA, blood glucose vs. group interaction was significant (p<0.001). Paired comparisons showed that DM vs. C and IH (p<0.001), and DM + IH vs. C and IH (p<0.001) were significant, indicating that glucose levels increased significantly in STZ-treated groups.

Table 4. The relative mRNA expression levels of HIF-1α, VEGF164 and VEGF188 (mean±SD) in four experimental groups

Genes	C	IH	DM	DM+IH
HIF-1α	0.41±0.20	0.40±0.10	0.43±0.18	0.46±0.16
$VEGF_{164}$	0.27±0.08	0.22±0.04	0.25±0.04	0.23±0.05
$VEGF_{188}$	1.08±0.25	0.96±0.15	0.90±0.10	0.77±0.14*

C - control; DM - diabetes mellitus; DM+ICH - diabetes mellitus + intermittent hypoxia; HIF-1 - hypoxia inducible factor-1 alpha; ICH - intermittent hypoxia; $VEGF_{164}$ - vascular endothelial growth factor 164; $VEGF_{188}$ - vascular endothelial growth factor 188. *p<0.05 vs. control

Weights

The weight measures of the animals from day 1 and day 57 along with their percentage changes are shown in Table 3. The percentages of weight change of the groups during the experiment (between day 1 and day 57) were statistically significant between DM vs. C and IH (p<0.001), DM+IH vs. C and DM (p<0.05), and DM+IH vs. IH (p<0.001) groups.

HIF-1α, VEGF164, and VEGF188 mRNA expressions

The relative mRNA expression levels of all experimental groups are shown in Table 4. Briefly, the left ventricle mRNA expressions of HIF-1α and VEGF164 were not significant among the groups (p>0.05). The only significance reached was in VEGF188 between the C and DM+IH groups (p<0.05).

Histological findings

Light microscopic examination of myocardial tissues

The myocardial structure was intact and no necrosis was observed in the control groups. In the DM group, however, the cardiac muscle fibers were disordered and the myocardium was disorganized along with vacuolization and necrosis. Myofibrillar damage and vacuolization were less persistent in the IH group. Additionally, there was vasodilatation, stasis, and congestion as well as increase in vascularization in the IH group. The DM+IH group had similar changes as those in the IH group but the tissue damage was much more obvious. Furthermore, the tissue destruction in the DM group was more evident than that in the DM+IH and IH groups (Fig. 5).

MVD

The average values from three separate observations from the experimental groups were compared and no statistical significance was reached (p>0.05, Table 5). Nevertheless, the IH group showed a 12% increase in capillarization compared with the control group. Moreover, the DM group had a 4.8% decrease in MVD values, whereas they were not different in the DM+IH group compared with the control group.

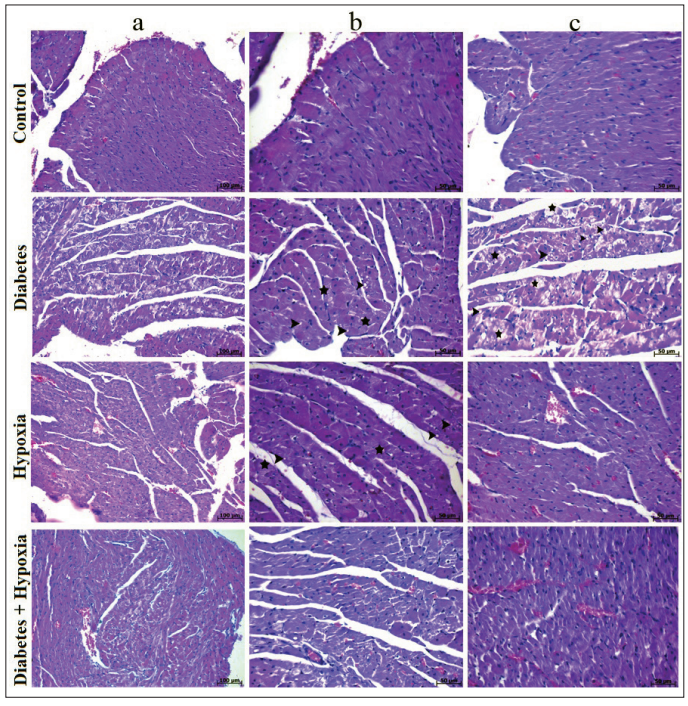

Figure 5. a-c. The histological light microscopy images of the left ventricle tissues stained with hematoxylin and eosin from three different animals (a, b, c) from each group.
The arrow head indicates vacuolization and the star indicates degenerated myofibril

Table 5. The microvascular density (#of capillary/unit area)

	C	IH	DM	DM+IH
Microvascular density	255.50±23.33	286.00±18.88	243.10±42.48	252.56±48.01

C - control; DM - diabetes mellitus; DM+IH - diabetes mellitus + intermittent hypoxia; IH - intermittent hypoxia. #Number

Discussion

The main findings of the present study are the hyperglycemia- and hypoxia-induced histological changes such as tissue organization and vascularization in the cardiac left ventricle. Briefly, the damage in the myocardium was most abundant in STZ-induced diabetic rats. DM+IH rats had less injury and only the IH rats had the least damage, which was close to normal. Vascularization was also increased in the two groups exposed to intermittent hypoxia. When the underlying molecular mechanisms were examined, there were no significant changes in the HIF-1α/VEGF pathway of the myocardium, except a decrease in $VEGF_{188}$ expression in the DM+IH group.

Diabetes is characterized mainly by hyperglycemia due to disturbances in either insulin secretion, insulin effects, or both. Type 1 diabetes is caused by the disruption of pancreatic beta cells. STZ, which is toxic to pancreatic beta cells, is used for developing experimental type 1 diabetes in animal models (20, 29). Following the STZ injection, hyperglycemia and weight loss are indicators for the diabetes. In STZ-treated groups, hyperglycemia was determined, which confirms the development of diabetes. Glucose levels of the DM and DM+IH groups are shown in Table 2. Moreover, we observed only 11% weight gain in diabetic rats comparing with 42% increase in untreated control rats in 8 weeks. The light microscopic findings of the left ventricle also prove the diabetes effect in the DM group. Diabetes has been shown to cause structural and functional disturbances in the myocardium through myocardial fibrosis, collagen formation, myocyte hypertrophy, mitochondrial dysfunction, and ROS accumulation (4). Tissue injury was observed in heart tissues of the DM group. The extent of tissue damage in diabetic rats exposed to intermittent hypoxia for 6 weeks (the DM+IH group) was reduced. Therefore, it can be suggested that IH attenuates diabetic myocardial damage. Without STZ treatment, intermittent exposure to hypoxia itself also created some disorganization in the myocardium, but this was not compatible with the diabetic changes. It has been shown that intermittent hypoxia accelerates the cellular adaptation response to stress and strengthens the antioxidant defense mechanisms as well as also improves cardiac function and reduces the ischemia-induced infarct size (7, 30). Furthermore, it has been reported that intermittent hypoxia increases myocardial capillarity, perfusion, and contractility and protects the myocardium from reperfusion injury by improving end-diastolic volume and function (8, 31, 32). Besides, high-altitude inhabitants have a lower cardiovascular morbidity and mortality (33). To the best of our knowledge, any alleviating effect of intermittent hypoxia on diabetic cardiac injury has not yet been investigated. One example showing the hypoxia-diabetes relation is a study from Peru, which resulted in low diabetes prevalence in high-altitude populations (34).

Diabetic cardiomyopathy was first recognized in diabetic patients with congestive heart failure who had no evidence of significant valvular, hypertensive, or coronary atherosclerotic disease and no other cause for cardiomyopathy (35). This specific diabetic heart disease is manifested by diastolic dysfunction at the beginning and has the risk for developing heart failure at the end, which becomes more apparent in the presence of other diabetic complications such as hypertension and/or myocardial ischemia (3, 4). Cardiac dysfunction is evident within 5 weeks after the STZ injection (36). Trost et al. (37) have showed a 15% decrease in left ventricular systolic pressure and 34% decrease in the maximum speed of relaxation following 3 weeks of STZ treatment in mice. Previously, we have applied 28 days of intermittent hypobaric hypoxia to control and diabetic rats and examined the functional parameters of the left ventricle. According to our unpublished data, left ventricular end-diastolic pressure (LVEDP) increased and left ventricular developed pressure (LVDP) decreased in diabetic rats comparing with the control rats. The contractility indexes and rates of the left ventricle pressure rise and decline (+dP/dt and -dP/dt) also decreased significantly. Intermittent hypoxia did not change the

functional parameters in the control group rats, but when applied to the diabetic rats, it restored cardiac function. This previous preliminary study proves that diabetes causes cardiac dysfunction and intermittent hypoxia application alleviates diabetic cardiomyopathy. In present study, cardiac tissue recovery from diabetic injury observed histologically consistent with functional improvement in our preliminary study.

Another outcome of the present study confirming the cardioprotective effect of IH is the change in MVD. As stated in the light microscopy and MVD results, we demonstrated that diabetes decreased, whereas IH increased the vascularity in the myocardium. When diabetic rats were exposed to intermittent hypoxia, the diminished MVD returned to control levels. Enhanced myocardial capillarity and associated increased perfusion by IH and their beneficial effect on ischemia tolerance have been reported in previous studies (8, 31, 32). One of the diabetic cardiomyopathy causative factors is compromised angiogenesis. Thompson et al. (38) have demonstrated decreased capillary diameter and density in STZ-treated rats, which returned to normal levels by the insulin treatment. The 26% decrease in MVD of diabetic mice in 5 weeks has found to be correlated with the systolic dysfunction (39). An inadequate angiogenic response and microvascular abnormalities in the myocardium of patients with diabetes could result in poor collateral formation that leads to an imbalance between myocardial supply and demand, thereby contributing to adverse cardiovascular events such as increased myocardial injury during ischemic events. Therefore IH-induced angiogenesis as seen in our study exerts a protective effect on a vulnerable diabetic heart against ischemic insults because of a reduced angiogenic response. Myocardial collateral formation is essentially regulated by VEGF, which has been shown to be downregulated in the diabetic myocardium in early studies (18, 19, 39). These authors suggested that both impaired angiogenesis and microcirculatory dysfunction in diabetics may be due in part to decreased expressions of VEGF and its receptors. We observed a slight decrease in both $VEGF_{188}$ and $VEGF_{164}$ isoforms in the STZ-treated groups compared with the control group, and statistical significance was reached only in $VEGF_{188}$ mRNA in the DM+IH group. The vascularization change observed in diabetic left ventricles could be caused by this finding. In fact, there are inconsistent reports in the literature regarding the effects of experimental diabetes on VEGF expression in the myocardium. It could be either high despite reduced neoangiogenesis (40) or low (18) or no change, as in the study by Broderick et al. (41). This may relate to the duration and severity of diabetes or to differences in downstream signaling of VEGF (42).

On the other hand, intermittent hypoxia did not affect VEGF expression in the left ventricle in the present study. Recently, we have shown that both acute and intermittent normobaric hypoxia increased the VEGF mRNA expression in the left ventricle in rabbits (15). The duration and intensity of exposed hypoxia as well as the animal species were different in the two studies. However, several other studies confirmed that the intermittent hypoxia induces myocardial angiogenesis by up-regulating VEGF (17, 43). Although the exact reason for these results in this study is unknown, an early transient increase in mRNA expression before processing the tissue might have led to the missing findings. A time-course evaluation of the mRNA and protein expressions of VEGF during the hypoxia treatment would be helpful to observe the potential changes. VEGF is one of the target genes of hypoxia-inducible factor-1α (HIF-1α), a transcriptional regulator complex that controls the expression of multiple hypoxia responsive factors (44, 45). It is well-known that HIF-1α has an important role in mediating intermittent hypoxia-induced angiogenesis and cardioprotection against ischemia/reperfusion injury (8-10). Therefore, its involvement in the intermittent hypoxia effect on diabetic cardiomyopathy is conceivable. We did not observe any alteration in cardiac HIF-1α mRNA expression neither following 6 weeks of intermittent hypoxia nor with the diabetic condition. However, we cannot exclude the critical role of the HIF-1α/VEGF pathway in this signaling event. As a matter of fact, parallel to our results, no change in endogenic HIF-1α mRNA expression has been found in diabetic rats, despite the reduced capillarity and VEGF mRNA expression reported by Xue et al. (46). Nevertheless, when they applied transgenic HIF-1α overexpression, they observed recovery from diabetes in terms of increases in VEGF expression and capillary density and a decrease in myocardial fibrosis. Nondiabetics did not show any difference in capillary density and mRNA and protein expressions of VEGF with HIF-1α overexpression, which was similar to our IH results (46). Meanwhile, in a recent study, Milano et al. (47) were unable to demonstrate HIF-1α/VEGF upregulation induced by intermittent hypoxia, despite the marked functional improvement and capillarity increase of the myocardium. Instead, the PI3K/Akt pathway was suggested to be involved in IH-induced cardioprotection in this study. Similarly, Rakusan et al. (48) reported VEGF-independent vascularization and cardioprotection induced by IH with the involvement of caveolin-1 and a receptor of angiotensin.

Study limitations

Along with the inconsistent data in the literature, to be assured for molecular mechanisms, we require further experiments including examination of protein and mRNA expressions of both the HIF-1α/VEGF pathway and other potential factors such as other angiogenic growth factors and VEGF receptors in a detailed time-course study for hypoxia treatment.

Conclusion

In this experimental study, we evaluated the myocardial effects of STZ-induced diabetes and of 6 weeks of mild systemic intermittent hypoxia in Wistar Albino rats. Left ventricle mRNA expressions of HIF-1α and VEGF were also examined to reveal

possible molecular mechanisms involved in these effects. The tissue organization and MVD of the left ventricle were affected both by hyperglycemia and the hypoxic stimulus. Although diabetes diminished the angiogenesis and VEGF expression, intermittent hypoxia treatment reversed this effect. Histological abnormalities induced by diabetes were also restored by hypoxia. The HIF-1α/VEGF pathway was not affected by intermittent hypoxia. The possible early and/or transient increase in HIF-1α mRNA levels could be overlooked and a protein analysis for both HIF-1α and VEGF is necessary for prospective studies.

Diabetic cardiomyopathy is a specific heart disease to diabetes and has been investigated extensively. Several pathophysiological courses and treatment strategies have been proposed till date. According to the results of the present study, intermittent hypoxia application has a promising potential for recovering both myocardial tissue injury and decreased angiogenesis. However, further detailed studies are warranted for elucidating the relevant molecular mechanisms and for the clinical translation of experimental findings.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

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