Chronic Intermittent Hypoxia Protects the Rat Heart Against Ischemic/Reperfusion Injury by Modulating Apoptosis: A Possible Role for Endogenous Nitric Oxide

Samah El-Attar
The Physiology Department, Faculty of Medicine, Cairo University

ABSTRACT

Background: *Intermittent hypoxia has been shown to provide myocardial protection against ischemia/reperfusion injury. Cardiac myocyte loss through apoptosis has been reported in ischemia/reperfusion injury. The role of nitric oxide (NO) in modulating apoptosis in rats exposed to chronic intermittent hypoxia is controversial. The aim of the present work is to investigate the possible role of nitric oxide synthase inhibition on modulation of apoptosis in ischemic- reperfused isolated hearts of rats exposed to chronic intermittent hypoxia. Methods: Adult male albino rats were used and exposed to normaxic or hypoxic conditions as follows: Group I: Normoxic conditions (normoxia group), Group II : Chronic intermittent hypoxia (CIH group) (10% O2 and 90% N2) for 8 hours daily, then to normal environmental air for the rest of the day, 5 days/ week for 4 weeks, Group III: Normoxic conditions and treated with L-NAME (10 mg/kg B.W. via intra-gastric route) (L-NAME group), Group IV: Chronic intermittent hypoxia and treated with L-NAME (CIH + L-NAME group) They had daily L-NAME(10 mg/kg B.W. via intra-gastric route) and exposed to the chronic intermittent hypoxia in the same way and duration as rats of group II. Isolated perfused hearts were subjected to 30 minutes of global ischemia followed by 30 minutes reperfusion. Left ventricular developed pressure (LVDP), contractility (dp/dt), and heart rate (HR) were recorded continuously. Expression of Bcl-2 in the myocardium was detected. Results: The parameters of functional recovery were improved in CIH group with significant increase in Bcl-2 expression as compared to normoxia group. Treatment with L-NAME led to attenuation of improved post–ischemic recovery of the ventricular function provided by chronic intermittent hypoxia with significant reduction in Bcl-2 expression compared with CIH group. Conclusion: adaptation to chronic intermittent hypoxia increases cardiac tolerance to ischemia/reperfusion. This protective effect was associated with increased expression of the antiapoptotic protein Bcl-2, that limits the apoptotic cell death in the myocardium following the ischemic/reperfusion insult. L-NAME attenuated both the improved recovery of cardiac function and the expression of antiapoptotic protein Bcl-2 induced by CIH.*

INTRODUCTION

Hypoxic states of the heart belong to the most frequent and dangerous diseases of modern time. They result from disturbed oxygen supply to cardiac cells, which is insufficient to meet their metabolic demands.[1]

Experimental and clinical studies have focused on the question of how cardiac tolerance to oxygen deprivation might be increased. [2] Adaptation to chronic hypoxia and various forms of preconditioning represent well defined and reproducible means to improve cardiac ischemic tolerance.[3] Chronic hypoxia, simulated in a barochamber, results in enhanced cardiac resistance in rats, but the precise mechanisms of this cardiac protection by adaptation to chronic hypoxia are still unclear.[4]

Apoptosis has been shown to contribute to myocardial reperfusion injury. Recent studies have demonstrated that myocardial ischemia and reperfusion result in apoptotic cell death, in addition to tissue necrosis.[5&6] However apoptosis does not become apparent until hearts are reperfused following an ischemic insult. [7] Webster et al.,[8] have demonstrated that in an in vivo model, both reperfusion, and hypoxia are strong stimuli for the induction of apoptosis. In fact, an increased rate of apoptosis has been observed in cerebral and cardiac reperfusion in animal models, suggesting that the deleterious effect during reperfusion is, at least in part, due to apoptosis.[9]

Although hypoxia may increase the incidence of apoptosis in cultured cardiomyocytes, the influence of chronic oxygen tension changes on apoptosis in cardiac cells in vivo is less clear. During hypoxia, there is cessation of mitochondrial oxidative phosphorylation, which normally fulfills the high metabolic needs of cardiomyocytes, and ATP is produced by much less efficient anaerobic glycolysis.[10] Immediate resumption of oxidative phosphorylation by reoxygenation, therefore, is critical for restoring adequate ATP production and cell survival. However, an abrupt rise in reactive oxygen species in mitochondria during reoxygenation has been associated with a deleterious effect on cardiomyocytes.[11]

Because the amount of myocardial damage in patients with ischemic heart disease is the most important determinant of morbidity and mortality, limiting the loss of cardiomyocytes during oxidative stress will have important therapeutic implications.[10] It has been suggested that, in reducing the apoptotic component within the ischemic area at risk, Bcl-2 (B-cell CLL/lymphoma 2) over-expression could lead to a ventricular function improvement.[12]

It is a well accepted concept that intracellular proteins of Bcl-2 family can be part of the apoptotic signaling cascade in which Bcl-2 exhibits antiapoptotic actions.[13] Bcl-2, a mammalian homologue of the antiapoptotic gene ced-9 in C elegans, is localized mainly to the mitochondrial membrane [14] and is a prototypical member of the Bcl-2 family of proteins that modulates apoptotic responses in various cell types.[15&16] Bcl-2 mRNA and proteins are expressed in developing and adult heart, [17] and the protein is upregulated after coronary occlusions.[18] However, the effects of Bcl-2 in heart have not been well characterized, and little is known about the effects of Bcl-2 in heart.[8]

Treatment with various NO-donating compounds is highly effective in the setting of myocardial I/R (MI/R) injury. NO has also

recently emerged as a crucial modulator of myocardial preconditioning, and NO is thought to mediate cardioprotection during the preconditioning process.[19]The role of nitric oxide (NO) in I/R injury and cardioprotection is complex and not fully understood. This molecule can increase cardiac ischemic tolerance by a number of cyclic GMP-dependent and independent mechanisms.[20]

Thus, a primary objective of the present study was to investigate the possible role of nitric oxide synthase inhibition on modulation of apoptosis in ischemic- reperfused isolated hearts of rats exposed to chronic intermittent hypoxia.

Materials and Methods:

Forty adult male albino rats weighing 200-250 gram were used in this study. They were divided into four groups;

Group I: Normoxia group, 10 rats were used as control rats and were exposed to normal atmospheric air.

Group II: Chronic intermittent hypoxia group, (CIH), 10 rats were used in this group and they were exposed to chronic intermittent hypoxia. The rats were placed in closed box and exposed to hypoxia (10% O2 and 90% N2) for 8 hours daily, then to normal environmental air for the rest of the day, 5 days/ week for 4 weeks[21]

Group III: Normoxia group treated with L-NAME (L-NAME group), 10 rats were used in this group. They had daily L-NAME in a dose of (10 mg/kg B.W. via intra-gastric route)[22] and were exposed to normal atmospheric air. Group IV: (CIH + L-NAME group) 10 rats were used in this group. They had daily L-NAME in a dose of (10 mg/kg B.W. via intra-gastric route)[22] and exposed to the chronic intermittent hypoxia in the same way and duration as rats of group II.

All rats had free access to water and fed on normal laboratory chow. They were exposed to 12-12 light-dark cycles placed in boxes at room temperature 23 C.

Langendorff Preparation:

Isolated heart perfusion was performed as described.[4] Sodium pentobarbital (45 mg/kg) was administered by intraperitoneal injection. The heart was excised, and the ascending aorta was cannulated and perfused at a constant pressure of 100 cm H_2O with Krebs-Henseleit buffer (in mmol/L, glucose 11, NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1.2, KCl 4.7, KH_2PO_4 1.2, and $MgSO_4$ 1.2), which was maintained at 37°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. A fluid-filled latex balloon was inserted into the left ventricle and inflated to an initial end-diastolic pressure of 4 to 8 mm Hg. Left ventricular developed pressure (LVDP), contractility (dp/dt), and heart rate (HR) were recorded continuously. Left ventricular developed pressure (LVDP) was calculated as the difference between the systolic and end-diastolic pressures.

Experimental protocol:

After a period of stabilization, baseline values of left ventricular developed pressure (LVDP), contractility (dp/dt) and heart rate (HR) were recorded. Then, an additional 5 min perfusion was carried out and values were again recorded. All hearts were then exposed to 30 min of sustained global ischemia

followed by reperfusion for 30 min. The values of LVDP, contractility and HR were measured at 10 min intervals during the reperfusion period, and were expressed as percentage of baseline value.

Detection of bcl2 gene expression by RT-PCR:

Gene expression of Bcl-2 was detected by reverse transcriptase polymerase chain reaction (RT-PCR). About 30 mg of heart tissue was homogenized then centrifuged at 14,000 rpm for 10 min. The supernatant was examined for detection of Bcl-2 expression.

RNA extraction:

RNA was extracted from tissue homogenate by acid guanidium thiocyanate, phenol chloroform. The RNA was reverse transcribed into cDNA by adding the following single strength RNA, PCR buffer, 5mM $mgcl_2$, 10mM dNTP (deoxy nucleotide triphosphate) mixture, 0.25ul reverse transcriptase and 2.5mMol random primer. The mixture was incubated at 42°C for 25 min then 5 min at 95°C then chilled on ice. The PCR was performed in a total of volume of 80 ml using specific primer of Bcl-2 : 5'GGGCTGAAAAGATTGGATCA3' and.
R:5'TCGAACAAATACCAGGAGC3'.

Taq polymerase (2.5ul), $mgcl_2$ (4mM), PCR cycling condition was performed for 40 cycles formed of 94°C for one minute , 55°C for one minute and 72°C for two minutes.

About 10ul of PCR product were electrophorised on 1.5% agarose gel stained with Ethedium Bromide and visualized by UV transilluminator, PCR products were semiquantitated using gel documentation system (BIODOC analyze) supplied by Biometra.[23]

Statistics

Values are measured as mean ± SD. Comparison of data was performed by using ANOVA test (analysis of variance test). Probability (P) values of <0.05 were considered to be significant.[24] Recovery was expressed as a ratio of post-ischemic value over the pre-ischemic value for LVDP, dp/dt and HR, thus each heart served as its own control.

RESULTS

Basal functional parameters of the perfused rat hearts (LVDP), (dp/dt), and heart rate (HR) are summarized in table1. There were no significant differences between the different rat groups.

Table (1): Left Ventricular developed pressure (LVDP) (mmHg), contractility (dp/dt) (cm/s), heart rate (beats/min) in different studied groups before induction of ischemia

	Normoxia group	CIH group	Normoxia+ L-NAME	CIH+ L-NAME
LVDP (mmHg) Mean ± S.D.	70.3±2.8	70.7±3.0	70.4±3.6	71.0±2.6
Dp/dt (cm/s) Mean ± S.D.	126.4±2.0	125.5±2.9	125.1±3.0	125.3±2.7
HR (beats/min) Mean ± S.D.	119.5±2.5	120.0±3.9	119.6±3.0	120.9±2.9
p		>0.05	>0.05	>0.05

Parameters of functional recovery of hearts in the different study groups:

After acquisition of baseline data, hearts were subjected to 30 min of global ischemia by halting the perfusion pump, followed by 30 min reperfusion. Upon reperfusion of hearts from rats exposed to normal atmosphere (G1), LVDP values immediately decreased to71%, the dp/dt were reduced to 69.8% and the heart rate values were reduced to 82.7%of the basal recorded values (Table 2, Figures1,2,3).

Table (2) shows that, in hearts exposed to 10% O2 for 8 hours daily for 4 weeks (CIH group), LVDP attained approximately 92.2% of the basal values after reperfusion with significant improved recovery compared to normoxia group(figure 1). CIH group also showed a better restoration of contractility that was 85.5% of the basal recorded values and was significantly increased as compared with normoxia group (figure 2). The heart rate was 91.6% restored after reperfusion with significant higher values as compared with normoxia group (figure 3). Also, it can be seen from figures 1,2,3 that the recorded values in the L-NAME treated normoxic group were not significantly changed as compared with normoxia group.

To determine whether the increased resistance to myocardial ischemia/reperfusion in hearts subjected to CIH was mediated by a mechanism involving NO or not, a group of rats were treated with L-NAME and exposed to chronic intermittent hypoxia for 4 weeks (CIH+ L-NAME). On reperfusion, LVDP attained 77.6% of the basal recorded values of this group, with significant reduction compared with CIH group. Furthermore, the contractility recovered only to75.2% of the basal recorded values versus 85.5% in CIH group. The heart rate was 84.3% restored at the end of reperfusion period with significant reduction as compared with CIH group. These values indicates a significant reduction in functional recovery of hearts from CIH+L-NAME group compared with values obtained from CIH group, although, there were a significant better recovery as compared with normoxia group(table 2, figures 1,2,3).

Table (2): Percent of recovery of left ventricular developed pressure (LVDP), contractility (dp/dt), heart rate (HR) in different studied groups after ischemia/ reperfusion.

	Normoxic group	**CIH group**	**Normoxia+ L.NAME**	**CIH+L-NAME**
Percent of recovery of LVDP(mmHg) Mean ± S.D.	71.0±2.2*	92.2±4.4º	72.4±4.5*	77.6±2.2*º
Percent of recovery of dp/dt (cm/s) Mean ± S.D.	69.8±2.1*	85.5±2.4º	68.8±1.8*	75.2±1.9*º
Percent of recovery of HR (beats/min) Mean ± S.D.	82.7±1.5*	91.6±4.2º	81.3±3.2*	84.3±1.7*º

ºSignificant change compared with normoxic group (P< 0.05).
** Significant change compared with CIH group (P< 0.05).*

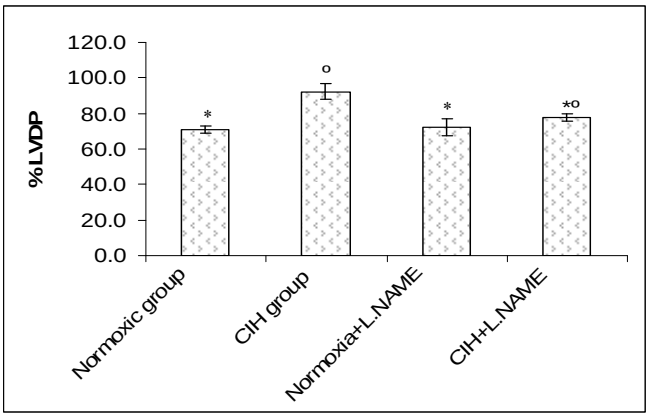

Fig. (1): Percent of recovery of left ventricular developed pressure (LVDP) (mmHg) after ischemia/reperfusion in different studied groups.
*ºSignificant change compared with normoxic group * Significant change compared with CIH group.*

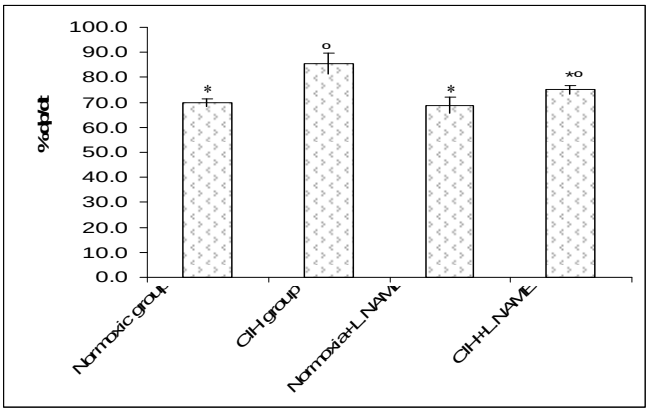

Fig. (2): Percent of recovery of contractility (dp/dt) (cm/s) after ischemia/reperfusion in different studied groups.

ºSignificant change compared with normoxic group Significant change compared with CIH group.*

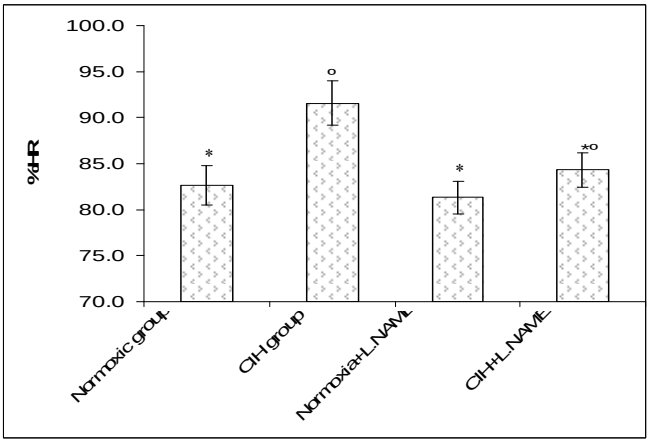

Fig. (3): Percent of recovery of heart rate(beats/min) after ischemia/reperfusion in different studied groups.

*ºSignificant change compared with normoxic group * Significant change compared with CIH group.*

Bcl-2 expression:

The quantification of Bcl-2 protein expression in protein homogenates from normoxic and hypoxic groups revealed that, Bcl-2 expression level was significantly up regulated in ischemically adapted hearts in the CIH group with mean value of 5.1±0.2 ng/mg of cardiac tissue compared with normoxia group(1.9±0.2 ng/mg of cardiac tissue). We next determined the extent

to which L-NAME affect the expression of Bcl-2 gene. It was found that in rats treated with L-NAME during the exposure to CIH (CIH+L-NAME group), Bcl-2 gene expression was 2.9 ± 0.2 ng/mg of cardiac tissue with significant reduction as compared with CIH group, however it was still significantly up-regulated as compared with normoxic group (table3, figure 4).

Table (4): Bcl-2 protein expression in heart cells in different studied groups.

	Normoxic group	**CIH group**	**Normoxia+ L.NAME**	**CIH+L.NAME**
Bcl-2 protein expression (ng/mg) Mean ± S.D.	1.9±0.2*	5.1±0.2º	1.8±0.2*	2.9±0.2*º

ºSignificant change compared with normoxic group.
** Significant change compared with CIH group.*

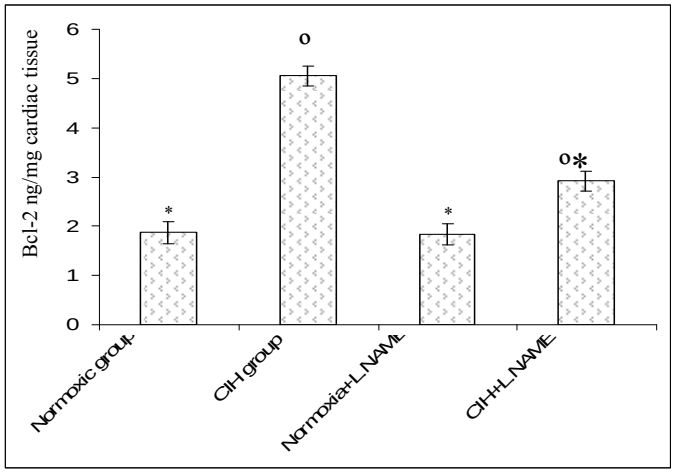

Fig. (4): Bcl-2 protein expression in heart cells in different studied groups
*ºSignificant change compared with normoxic group * Significant change compared with CIH group*

DISCUSSION

The mammalian heart can be adapted to ischemia by repeatedly subjecting it to short-term reversible ischemia followed by short durations of reperfusion or by intermittent hypoxia. Such adaptation, generally known as preconditioning (PC), is cardio-protective as evidenced by its ability to reduce myocardial infarction, tissue injury, arrhythmias, and to improve post-ischemic ventricular functions.[6]

In the present study, adult rats exposed to 4 weeks of intermittent

hypoxia had improved post ischemic left ventricular functions, as evidenced by significantly better recovery of LVDP, dp/dt, and HR, compared to normoxic group.

The majority of studies demonstrated that the hearts of adult chronically hypoxic animals exhibits better functional recovery following ischemia as compared to controls.[2] Cai et al. [25] and Baker et al.[26] demonstrated that adaptation to hypoxia increased the tolerance of adult rat hearts or of rabbit hearts on the 4th day of postnatal life to ischemic reperfusion injury.

As cardiac protection against acute I/R injury induced by chronic hypoxia is an important form of preconditioning and lasts markedly longer than any other form of preconditioning, [2] therefore its molecular mechanism is of particular interest for potential clinical application in the future. However, chronic hypoxia has been much less studied, and the understanding of its protective signaling is still limited. [27]

Preconditioning consisting of one or more episodes of ischemia/reperfusion or by episodes of hypoxia and normoxia causes the development of oxidative stress, Inhibition of oxygen free radical by antioxidants abolishes cardioprotection offered by chronic intermittent hypoxia. This is only possible if the reactive oxygen species potentiate the signal transduction cascade leading to preconditioning (PC). [28]

However, the amount of oxidative stress is not cumulative for each subsequent episode. The amount of oxidative stress generated lessens during each subsequent episode. During prolonged ischemia and reperfusion, the amount of oxidative stress is actually lower in the PC myocardium compared with non-PC hearts. [29]

Cellular necrosis inevitably follows extended periods of anoxia (i.e., oxygen absent) or severe hypoxia (i.e., oxygen supply decreased relative to metabolic demand). Hypoxic tolerance of various cell types differs, depending on the metabolic rate and intrinsic adaptive mechanisms of the tissue. Sublethal hypoxia, which may be transient and have no apparent consequences, can be followed by enhanced resistance to reoxygenation injury (conditioning). Post-hypoxic injury is due to a combination of changes in cellular energy charge, oxidant generating systems, and antioxidant defenses.[30]

Maulik et al.[28] have demonstrated that acute myocardial ischemia and reperfusion resulted in apoptotic cell death, in addition to necrosis. Another study showed that PC provided cardioprotection by blocking apoptotic cell death.[6]

Oxidative stress developed in the ischemic reperfused myocardium was found to be instrumental for apoptotic cell death, because free radical scavengers were found to block apoptotic cell death simultaneously, providing myocardial protection[8]. So decreasing the amount of oxidative stress by exposure to chronic intermittent hypoxia, leads to reduction of apoptotic insult.

Other related studies suggested that prolonged reperfusion after ischemia caused down regulation of

the antioxidant gene, Bcl-2, in concert with enhanced DNA fragmentation. The protective effect of chronic hypoxia may be related to enhanced Bcl-2 expression. [31]

Chronic hypoxia increases expression of hypoxia-inducible factor 1 (HIF-1) in the rat myocardium that mediates adaptive expression of other potentially protective proteins. [27] Several oxidative stress-inducible genes become activated during PC, of which Bcl-2 appears to be the most important gene that inhibits apoptosis.[32]

The second finding in our study supports this suggestion. It was found that, rats exposed to chronic hypoxia had significantly higher rate of myocardial expression of antiapoptotic factor Bcl-2 as compared with normoxic rats.

Similar results were reported by Dougherty et al., [13] who suggested that exposure of isolated cardiac myocytes to chronic hypoxia is followed by activation of c-JUN- N-terminal Kinase (JNK) which promotes survival of myocytes after oxidative stress and they suggested that Bcl-2 family proteins are targets for phosphorylation by JNK.

Also, Maulik et al.[28] suggested an inverse relation between cardiomyocytes apoptosis and the induction of the antioxidant gene, Bcl-2.

Bcl-2 may be regarded as an important cellular component that not only guards against apoptotic cell death but also impinges on multiple cellular events. Expression of Bcl-2 gene was found to be associated with inhibition of apoptosis mediated by multiple agents; Ca2+ ionophore, glucose withdrawal, membrane peroxidation, and free radical injury, suggesting that this gene is likely to play a role in reperfusion injury. [33]

The ratio of the antiapoptotic protein, Bcl-2, and the proapoptotic protein, Bax, seems to be critical for cell survival.[34] The activities of Bcl-2 proteins are regulated by phosphorylation. Phosphorylation may activate or inactivate the antiapoptotic function of Bcl-2 family proteins in a manner that is determined by the protein targets and the specific residue phosphorylated.[27]

One mechanism by which Bcl-2 prevents cell death during physiological and pathophysiological processes is through the inhibition of mitochondrial cytochrome C release. A reduction of Bcl-2 leads to an increase of Bax homodimers resulting in translocation of Bax to mitochondrial membrane with subsequent mitochondrial dysfunction that leads to loss of membrane potential and release of cytochrome C into the cytosol. Cytochrome C then forms a complex with apoptosis-activating- factor, activating caspase 9, which triggers a proteolytic cascade leading to apoptotic cell death. [35]

Mitochondria from apoptotic cells produce increased quantities of O_2^- because of a switch from normal four-electron reduction of O_2 to one-electron reduction after cytochrome C release. Over-expression of the antiapoptotic protein Bcl-2 prevents increased O_2^- production associated with apoptosis. [36]

The third finding in the present study is that L-NAME treatment had no significant effect on the post

ischemic/reperfusion ventricular function recovery or on myocardium expression of Bcl-2 proteins in hearts of normoxic rats. On the other hand, L-NAME treatment significantly attenuated the improved functional recovery of ventricular function and the enhanced expression of Bcl-2 proteins recorded in heart of rats exposed to chronic intermittent hypoxia.

Our results are in accordance with results of Baker et al., [26] and Fitzpatrick et al.[37] who showed that acute inhibition of NOS activity by L-NAME led to complete abolition of improved post ischemic recovery of contractility by chronic hypoxia in adults and neonatal rats.

However, Szarszoi et al.[38] demonstrated that L-NAME had no effect on the improvement of post ischemic recovery of contractility in isolated, perfused hearts of hypoxic rats. On the other hand, Jones et al., [19] reported that endogenously formed NO significantly contributes to ischemia reperfusion injury. This controversy may be due to different protocols, and animal species.

The results of the present work suggested that nitric oxide is an important mediator of adaptation to chronic hypoxia, as inhibition of NOS activity by L-NAME led to attenuation of improved post–ischemic recovery of the ventricular function provided by chronic hypoxia.

Previous studies showed that chronic hypoxia from birth increased NOS activity. They found that nitrite plus nitrate content (an index of NOS activity) was elevated in chronically hypoxic hearts**.** [39]

Other studies demonstrated that chronic hypoxia increased eNOS levels, gene expression, and activity in adult rats**.** [37] Chronic hypoxia increases HIF-1 in the rat myocardium that mediates expression of NOS[25]. Also, chronic hypoxia increased basal myocardial NO production by activation of constitutive NO synthase due to down regulation of caveolin-3**.** [40]

Several lines of evidence suggested that during reperfusion after ischemia, there is excess production of NO through elevation of intracellular Ca^{2+}, which stimulates Ca^{2+} dependent NOS in cardiac myocytes. Also reintroduction of molecular oxygen following ischemia favors generation of NO**.** [41&42]

This increased NO is suggested to attenuate G1 cyclins and associated cyclin dependent kinases thought to mediate apoptosis in hypoxic cells**.** [43]

Generally the anti-apoptotic effects of NO can be mediated through a number of mechanisms such as the nitrosylation and inactivation of many of the caspases including caspase 3, caspase 1 and caspase 8.[35] Other mechanisms include activating p53, upregulating heat shock protein 70 (and consequently blocking recruitment of pro-caspase 9 to the Apaf-1 apoptosome)**.** [44]

It has been proposed that endogenous NO plays a positive role in increased ischemic tolerance of chronically hypoxic rabbit hearts by a mechanism which involves activation of soluble guanylyl cyclase, accumulation of cyclic GMP, possible activation of cGMP-dependent protein kinase and phosphorylation of mitochondrial K-ATP channels**.** [37]

The molecular mechanisms underlying the NO/cGMP inhibition of apoptosis could involve the activation of cGMP-dependent protein kinase and the inhibition of caspase activation. However, the precise mechanism by which cGMP or PG kinase suppresses apoptotic signaling remains unknown.

Although endogenous inhibitors of caspase activation and activity have been described, none has been shown to be more prevalent than NO. An important feature of NO inhibition of caspase activity is that NO can rescue a cell from apoptosis even after the caspase cascade has been activated. Because NO easily diffuses within a cell, as well as from cell to cell, NO can efficiently guard against aberrant activation of caspases**.** The inhibition of caspase activation may then limit Bcl-2 degradation, [(35)] and thus explain the increase in Bcl-2 levels observed.

NO can oxidize intracellular reduced glutathione and thereby change the antioxidant levels within the cell, resulting in oxidative or nitrosative stress. This action stimulates the induction of heat shock proteins HSP32 (heme oxygenase) and HSP70, which protect cells from apoptotic cell death. [(45)]

Moreover, the accumulated data indicate that physiologically relevant levels of NO contribute to the balance between the antiapoptotic and proapoptotic forces within a cell by suppressing the apoptotic pathway at multiple levels and by several mechanisms. Higher rates of NO production overwhelm cellular protective mechanisms and shift the balance toward apoptotic death in some cell types. [(35)]

Some studies suggest that NO has a proapoptotic effect as a result of the formation of peroxynitrite, inducing apoptotic DNA fragmentation and p53-dependent apoptosis. The formation of peroxynitrite is determined by the ratio of NO to superoxide, and the cellular susceptibility to peroxynitrite is dependent in large part on the levels of antioxidants (eg, thiols)**.** [(46)]

However, beside its toxic effect, peroxinitrite is considered an important upstream event triggering mechanism of late preconditioning. [(47)] Also it has been suggested that, the association of eNOS with heat shock protein 90, produced in stressful conditions, helps to produce NO and to limit superoxide generation. [(44)]

Conclusion:

It may be concluded that adaptation to chronic intermittent hypoxia increases cardiac tolerance to acute oxygen deprivation and reperfusion as shown by better recovery of cardiac function. This protective effect was associated with increased expression of the antiapoptotic protein Bcl-2 that limits the apoptotic cell death in the myocardium following the ischemic/ reperfusion insult.

It appears that increased synthesis of NO is one of the mechanisms by which chronic intermittent hypoxia offer this protection; since inhibition of NO production by L-NAME attenuated both the improved recovery of cardiac function and the expression of antiapoptotic protein Bcl-2 induced by CIH.

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الملخص العربي

سماح العطار
قسم الفسيولوجى- كلية الطب – جامعة القاهرة

أثبتت الأبحاث أن نقص الأكسجة المتقطع يزوّد القلب بحماية ضد الاصابات الناتجة من حالة فقر دم الاحتباسيّ ثم عودتها الى طبيعتها،كما أثبت أنه يحدث فقدانا للخلايا عن طريق الموت المبرمج في حالات الاصابات الناتجة من نقص الدورة الدموية للقلب ثم عودتها الى طبيعتها. ويلاحظ أن دور أكسيد النيتريك في التأثير على الموت المبرمج للخلايا في حالات نقص كمية الأكسجين المتقطع المزمنة غير واضح.

الهدف من هذه الدراسة هو أن تتحرّى امكانية وجود دور لمثبطات الانزيم المصنع لأكسيد النيتريك في التأثير على الموت المبرمج لخلايا القلوب المعرضة لنقص الدورة الدموية للقلب ثم عودتها الى طبيعتها في الفئران التي سبق تعريضها لنقص كمية الأكسجين المتقطع المزمنة.

استخدمت ذكور فئران بالغة في هذه الدراسة و تم تقسيم الفئران الى أربع مجموعات:

المجموعة الأولى: شملت عشرة فئران تعرضت للنسبة الطبيعية من الأكسيجين ثم تعرضت الى حالة فقر دم الاحتباسيّ ثم عودتها الى طبيعتها.

المجموعة الثانية:تعرضت لحالة نقص أكسجة مزمنة متقطّعة (١٠% اكسجين و ٩٠%نيتروجين لمدة ٨ ساعات يوميّة، بعد ذلك إلى هواء عادى بقية اليوم، ٥ أيام / أسبوع لمدة ٤ أسابيع..

المجموعة الثالثة: تعرضت للنسبة الطبيعية من الأكسيجين مع اعطائها ل–ن ا م ي (١٠ مغ/كغ وزن) عن طريق المعدة.

المجموعة الرابعة:تعرضت الى حالة نقص أكسجة مزمنة متقطّعة مع اعطائها ل–ن ا م ي (١٠ مغ/كغ وزن) عن طريق المعدة.

عرضت القلوب المعزولة إلى ٣٠ دقائق من حالة فقر دم احتباسيّ شاملة اتبعت ب ٣٠ دقيقة استعادة امرار المحلول. سجّل ضغط البطين الأيسرّالمتطوّر، قابليّةالانقباض، و عد النبضات باستمرار.و تم قياس انتاج ال [ب.سي.ل-٢] في عضلة القلب.

كانت النتيجة أن تحسنت المؤشرات الوظيفيّة في المجموعة التي تعرضت لحالة نقص أكسجة مزمنة متقطّعة مع زيادة هامّة إحصائيا في انتاج [ب.سي.ل-٢] مقارنة بالمجموعة الأولى أدت المعالجة ب [ل-ن.ا.م.ى] إلى تخفيف من التحسن في وظائف القلب بعد اعادة امرار المحلول الملحوظ في المجموعةالتي تعرضت لنقص أكسجة مزمنة متقطّعة مع تخفيض هامّ احصائيا في انتاج[ب.سي.ل-٢].

الاستنتاج:

يزيد تكييف القلب لحالة نقص الأكسجة المتقطّعة المزمنة من تحمل القلب إلى حالة فقر دم احتباسيّ ثم اعادة امرار المحلول. صحبت هذا التأثير الواقي زيادة إنتاج [ب.سي.ل-٢]، واستطاع أنّ يحدّ من فقدان الخلايا عن طريق الموت المبرمج وأن استخدام ال[ل-ن.ا.م.ى] أضعف التحسن الملحوظ في وظائف القلب والزيادة الملحوظة في انتاج ال[ب.سي.ل-٢].