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A POSSIBLE CARDIO-PROTECTIVE ROLE OF EARLY REMOTE ISCHEMIA PRECONDITIONING AGAINST ISCHEMIA REPERFUSION INJURY IN ISOLATED HEARTS OF ADULT ALBINO RATS: POSSIBLE INVOLVEMENT OF HYPOXIA INDUCIBLE FACTOR

By

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ABSTRACT

Background: In spite of the claimed cardio-protective effects of ischemic preconditioning (IPC), it is invasive and so using remote ischemic preconditioning (RIPC) may offer an alternative. Meanwhile, RIPC cardio protective role is controversial, with an equivocal underlying mechanism. The hypoxia inducible factor 1 alpha (HIF-1-alpha) which is increased following ischemic insults is claimed as a humoral mediator for RIPC.

Objectives: To investigate the effect of remote ischemic pre-conditioning on myocardial ischemia/reperfusion injury in rats, and to elucidate the possible role of hypoxia inducible factor in this protection.

Patients and Methods: The present study was performed on 28 adult female albino rats in the same estrus cycle evaluated by vaginal smear, and they were allocated into 3 groups: Group I: control rats subjected to ischemic /reperfusion injury (I/R) only, group II: early RIPC rats (RIPC 2 hours prior to I/R), group III: acriflavine-treated early RIPC rats. Acriflavine is a drug that binds directly to HIF-1 alpha and HIF-2 alpha subunits, thus inhibiting its dimerization and transcriptional activity, and it was injected IP 10 days prior to RIPC. On sacrifice day, ECG was recorded and isolated heart studies were performed. Later, cardiac chambers weight, serum HIF-1-alpha, myocardial perfusate lactate dehydrogenase, and cardiac oxidative markers: Malonaldehyde and glutathione perioxidase were measured.

Results: Compared to the control group, the early RIPC group showed significant increase in the heart rate (HR), QTc interval in the ECG recording, glutathione peroxidase and the HIF 1α levels together with reduction in the percent of decrease in PT and PT/LV, in the percent of prolongation in time to peak tension (TPT), perfusate lactate dehydrogenase and MDA levels, while no significant changes were recorded in the heart chronotropic activity, in the percent of half relaxation time (HRT) prolongation, or in the percent of decrease of MFR. Following acriflavine treatment, the effects of RIPC were abolished highlighting the role of HIF-1-alpha in mediating RIPC protective effects.

Conclusion: The non-invasive and non-pharmological remote ischemic preconditioning technique can ameliorate the cardiac ischemic reperfusion injury with an obvious role of HIF-1α in mediating these protective effects.

Key words: Cardiac, remote ischemic preconditioning, ischemic reperfusion injury, Acriflavine, HIF-1α, Peak tension.

INTRODUCTION

Cardiovascular diseases and ischemic heart diseases (IHD) have been considered as a major contributor to the total morbidity and mortality worldwide *(Candilio and Hausenloy, 2017).* Coronary heart disease (CHD) is responsible for about one-third of all deaths over age 35 worldwide *(Gomar et al., 2016).*

Ischemic preconditioning (IPC) is an experimental technique in which tissues exposure to brief episodes of ischemia protect them against a subsequent ischemic insult *(Hausenloy and Yellon, 2016).*

Cardiac ischemic preconditioning was able to reduce myocardial infarct size and to attenuate the incidence and the severity of reperfusion-induced arrhythmias *(Iliodromitis et al., 2007 and Heusch et al., 2015).*Classic IPC is invasive and can lead to dangerous complications including coronary artery rupture *(Zhang et al., 2013).* Remote ischemic-preconditioning (RIPC) has emerged as an interesting alternative to direct IPC *(Lemarroy, 2014).*

Remote ischemic preconditioning (RIPC) is a technique in which a remote organ as the limb is subjected to brief cycles of limb ischemia and reperfusion in order to confer protection of another target organ during subsequent ischemia *(Herrmann, 2010, Cai et al., 2013, and Gedik et al., 2017).* This protection can be provided in an early immediate phase that vanishes within 4 h and late phase that presents 24 h after the preconditioning stimulus and lasts for at least 48 h *(Vasdekis et al., 2013). Herrmann (2010)* suggested that RIPC is clinically appealing, being non-pharmacologic, non-invasive, quickly administered and may potentially protect multiple organs.

RIPC induction by left femoral artery occlusion reduces myocardial infarct size in the mice *(Lim et al., 2010)*. In addition, *Thielmann et al. (2013)* stated that patients who underwent RIPC before coronary artery bypass grafting (CABG) surgery displayed marked reduction in all cardiac causes of mortality. Similarly, RIPC in the upper limb in patients of acute myocardial infarction resulted in 27% reduction in infarct size *(White et al., 2014)*.

Meanwhile, *Hausenloy et al (2015) and Zaugg & Lucchinetti (2015)*; claimed that remote ischemic preconditioning fails to provide protection in patients undergoing cardiac surgery. *Zaugg and Lucchinetti (2015)* suggested that anesthetics may interfere and inhibit the protective effect of remote ischemia-induced preconditioning.

Moreover, the underlying mechanisms of this technique remain elusive. It has been hypothesized that RIPC predominantly involves systemic multi-factorial anti-inflammatory, neuronal, and humoral signaling pathways, that can be transferred to the target organs to offer their protection against ischemic injuries *(Ghani et al., 2017).Lee et al. (2009)* highlighted the importance of hypoxia

inducible factor 1α (HIF-1α) molecule as a major adaptive response to hypoxia.

In normoxia, HIF-1 α protein has a short half-life (t1/2- 5 min) as it is rapidly degraded *(Masoud and Li, 2015)*. Meanwhile, during ischemic insults or hypoxia, the half life of HIF-1 α increases up to 60 min *(Xiong & Liu, 2017 and Yang et al., 2018)*.

Therefore, this study was planned to assess the effectiveness of remote ischemic preconditioning in reducing myocardial ischemia/reperfusion injury in rats and to elucidate the possible role of hypoxia inducible factor in this protection.

MATERIALS AND METHODS

This study was performed on 28 adult female albino rats weighing 180-200 g at the start of the study. Rats were purchased from Helwan animal house and were kept at the Medical Ain Shams Research Institute (MSRI), Ain Shams University. Animals were housed in animal cages (50×30×20) cm each cage contain 5 rats, with suitable ventilation, temperature of 22-25°C and normal dark/light cycle. Food and water access were ad libitum. All rats were treated in accordance with the Guide for Care and Use of Laboratory Animals, and the study protocol was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University.

Rats were initially weighed and randomly distributed into three equal groups:

Group I (Sham-operated control group): Control rats that were subjected to ischemic /reperfusion injury.

Group II (Early RIPC group): Rats were subjected to RIPC 2 hours prior to ischemic /reperfusion injury *(Tork et al., 2015)*.

Group III (Acriflavine-treated early RIPCgroup): Rats were injectedwith Acriflavine, the HIF-1alpha inhibitor (IP, 2mg/kg/day) for 10 days prior to RIPC and 2 hours prior to ischemic /reperfusion injury *(Lee et al., 2009)*.

Remote ischemic preconditioning technique:

As described by *Tork et al (2015)*, rats were subjected to 3 cycles of ischemia/reperfusion. Each cycle consisted of 5 minutes of bilateral hind limb ischemia, followed by 5 minutes of reperfusion. RIPC was done using a rubber band tourniquet that was wrapped around both hind limbs to induce femoral artery ischemia. Ischemia was assured by the appearance of cold and cyanotic skin. RIPC was done either 2 or 24 hours before sacrifice according to the allocated group.

Rats were assigned to the different groups then, *on the sacrifice day*, overnight fasted rats were weighed, injected with 5000 IU/Kg B.W. heparin sodium (I.P.) *(Nile Company, Egypt)*, and then were anesthetized with I.P. injection of thiopental sodium *(EIPICO, Egypt)*, in a dose of 40 mg/kg B.W. ECG tracing was recorded. Blood samples were collected and stored at -80○C for later determination of serum hypoxia inducible factor- 1 alpha (HIF-1α).In vitro study of isolated hearts perfused in a Langendorff preparation was performed to record the intrinsic activity of heart under baseline condition and its response after 5,15,30 minutes of reperfusion following 30 minutes of total global ischemia using

isometric force transducer (UGO BASILE) connected with a USB cable to a recorder and to a computer provided with I Worx LabScribe2 Data Recording and Analysis Software. The MFR was assessed by collecting the fluid for 3 min. Cardiac tissues were weighed using 5-Digit-Metler balance (AE 163), then stored at -80 ℃ to assess Malondialdehyde (MDA) levels and Glutathione peroxidase (Gpx) enzymatic activity.

Biochemical analysis:

Serum HIF-1α was measured by ELISA using kits supplied by *YL Biont company*, according to *Wu et al. (2017)*. Both myocardial perfusate Lactate dehydrogenase and cardiac glutathione peroxidase were measured by a UV method described by *Paglia and Valentine (1967)*, using kits supplied by *Linear chemicals company* and Bio-diagnostic, Egypt respectively. Cardiac tissue MDA was measured by Thiobarbituric Acid (TBA) Testaccording to *Esterbauer and Cheeseman (1990)* using kits provided by Sigma Diagnostics, USA.

The results were expressed as mean ± one standard error and were statistically analyzed .Used *SPSS program version 20* $P \leq 0.05$ was considered significant , e.g : one way ANOVA with posthoc test (LSD), student-t test for paired data ,correlation studies and percent change.

RESULTS

Biochemical measures:

As shown in table 1, Early RIPC rats showed significant increase in the serum HIF 1α and significant decrease in MDA level compared to the control group. Acriflavine treatment caused a significant decrease in serum HIF 1α, and a significant increase in both MDA and LDH levels compared to the control group (Table 1).

Table (1): Serum HIF 1α (ng/ml), LDH (U/L), MDA (µM/g) and GPX (U/g) in all studied groups

Groups / Parameters	Control group (n=8)	Early RIPC group (n=11)	Acriflavine treated early RIPC group (n=9)
HIF 1α(ng/ml) Mean ± SEM	0.89 ± 0.13	1.44 ± 0.16	0.57 ± 0.14
a		<0.01	>0.05
b			<0.001
LDH (U/L) Mean ± SEM	183.04 ± 56.94	34.01 ± 7.252	243.43 ± 67.79
a		>0.05	>0.05
b			<0.01
MDA (µM/g) Mean ± SEM	1.26 ± 0.15	0.84 ± 0.12	1.25 ± 0.16
a		<0.05	>0.05
b			<0.05
GPx (U/g) Mean ± SEM	16.33 ± 4.11	24.66 ± 4.50	11.88 ± 2.78
a		>0.05	>0.05
b			<0.05

a: Significance of difference from the control group calculated by LSD at $P < 0.05$.

b: Significance of difference of acriflavine- treated early RIPC group from early RIPC group calculated by LSD at $P < 0.05$.

HR and QTc interval significantly increased in the early RIPC group compared to the control group. Meanwhile, they significantly reduced in acriflavine-treated early RIPC compared to the early RIPC group (Figure 1 a, b).

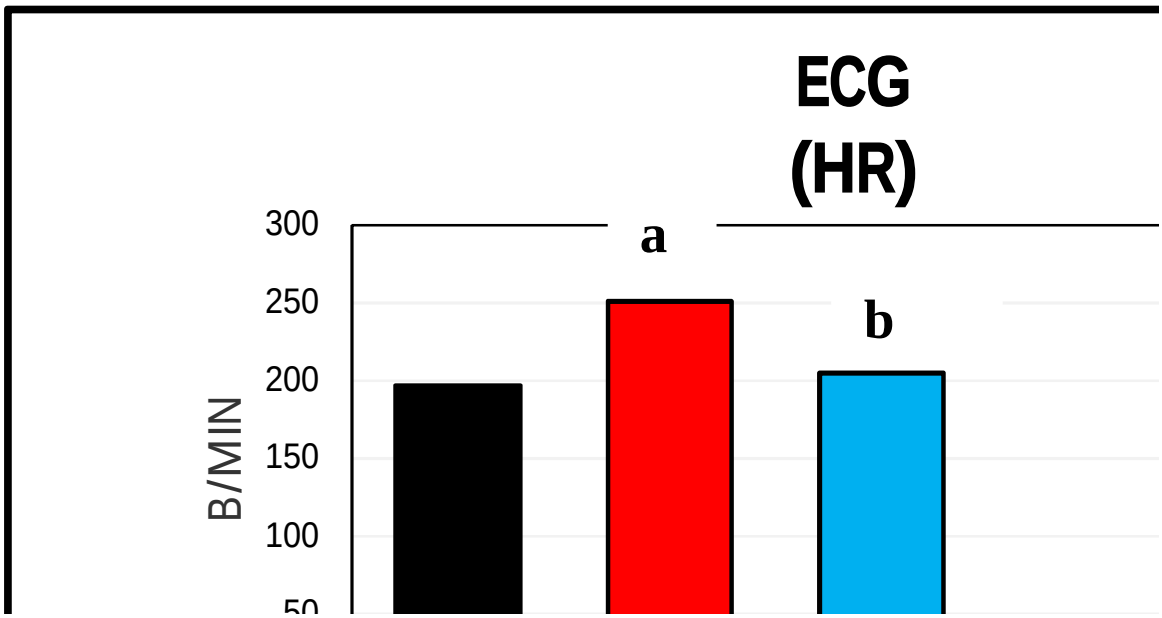


Figure (1a): Electrocardiographic changes heart rate in the 3 studied groups.

a: Significance of difference of treated group from the control group calculated by LSD at $P < 0.05$.

b: Significance of acriflavine treated early RIPC group from early RIPC group calculated by LSD at $P < 0.05$.

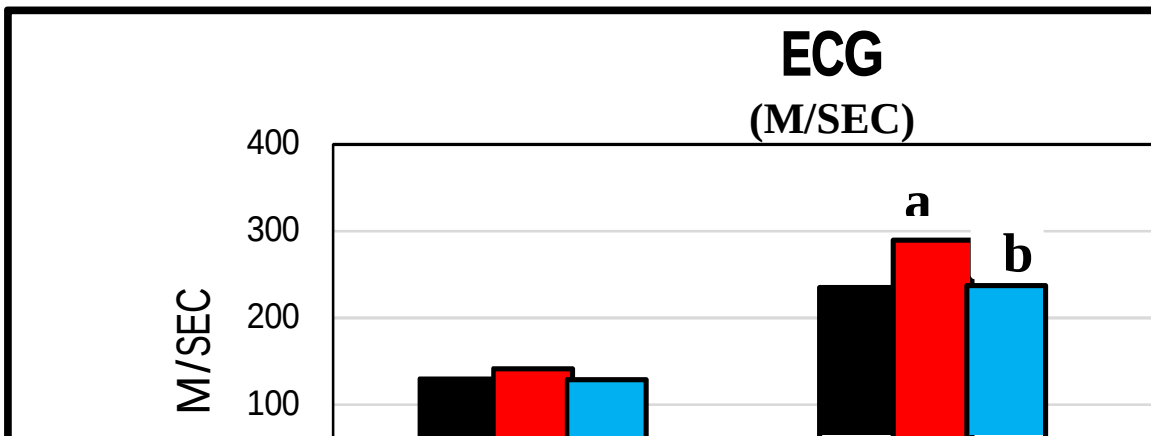


Figure (1b): Electrocardiographic changes (heart rate, Q-To interval and Q-Tc interval) in the 3 studied groups.

a: Significance of difference of treated group from the control group calculated by LSD at P < 0.05.

b: Significance of acriflavine treated early RIPC group from early RIPC group calculated by LSD at P < 0.05.

All reperfusion values significantly decreased after 5, 15 and 30 min of reperfusion compared to their baseline values in all groups (Figures2-6).

Regarding heart chronotropic activity, the percent of decrease in HR was insignificantly different in the early RIPC compared to the control group and significantly increased in acriflavine-treated early RIPC group after 30 min compared to the early RIPC group (Figure 2).

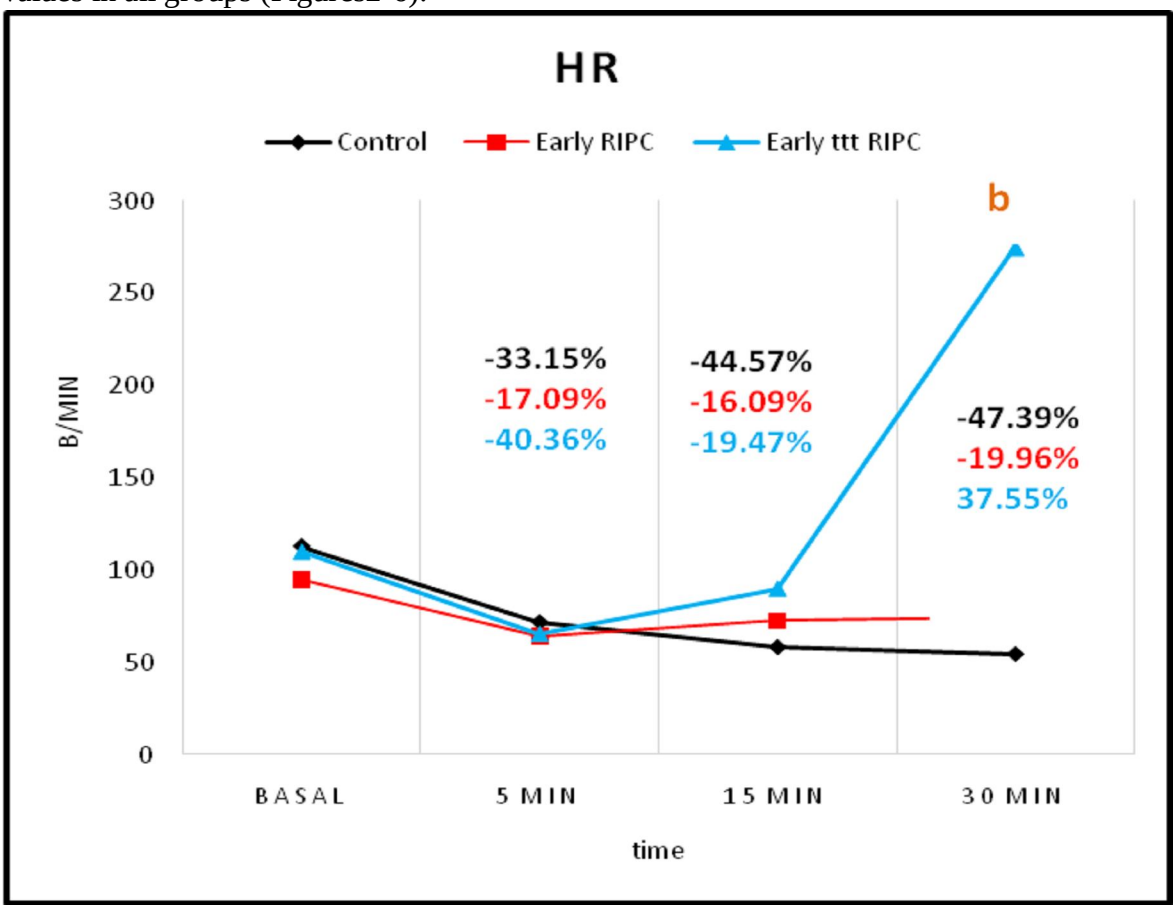


Figure (2): Heart rate preischemia baseline value and responses following ischemia and 30 minutes of reperfusion of hearts isolated from the control,early RIPC and acriflavine treated early RIPC groups.

a: Significance of difference of percent change from the control group calculated by LSD at P < 0.05.

b: Significance of difference from early RIPC group calculated by LSD at P < 0.05.

Compared to the control group, the percent of decrease in PT and PT/LV were significantly decreased in early RIPC group, meanwhile, they were significantly increased in the acriflavine treated early RIPC group compared to the early RIPC groups after 5, 15 and at 30 min in (Figure 3).

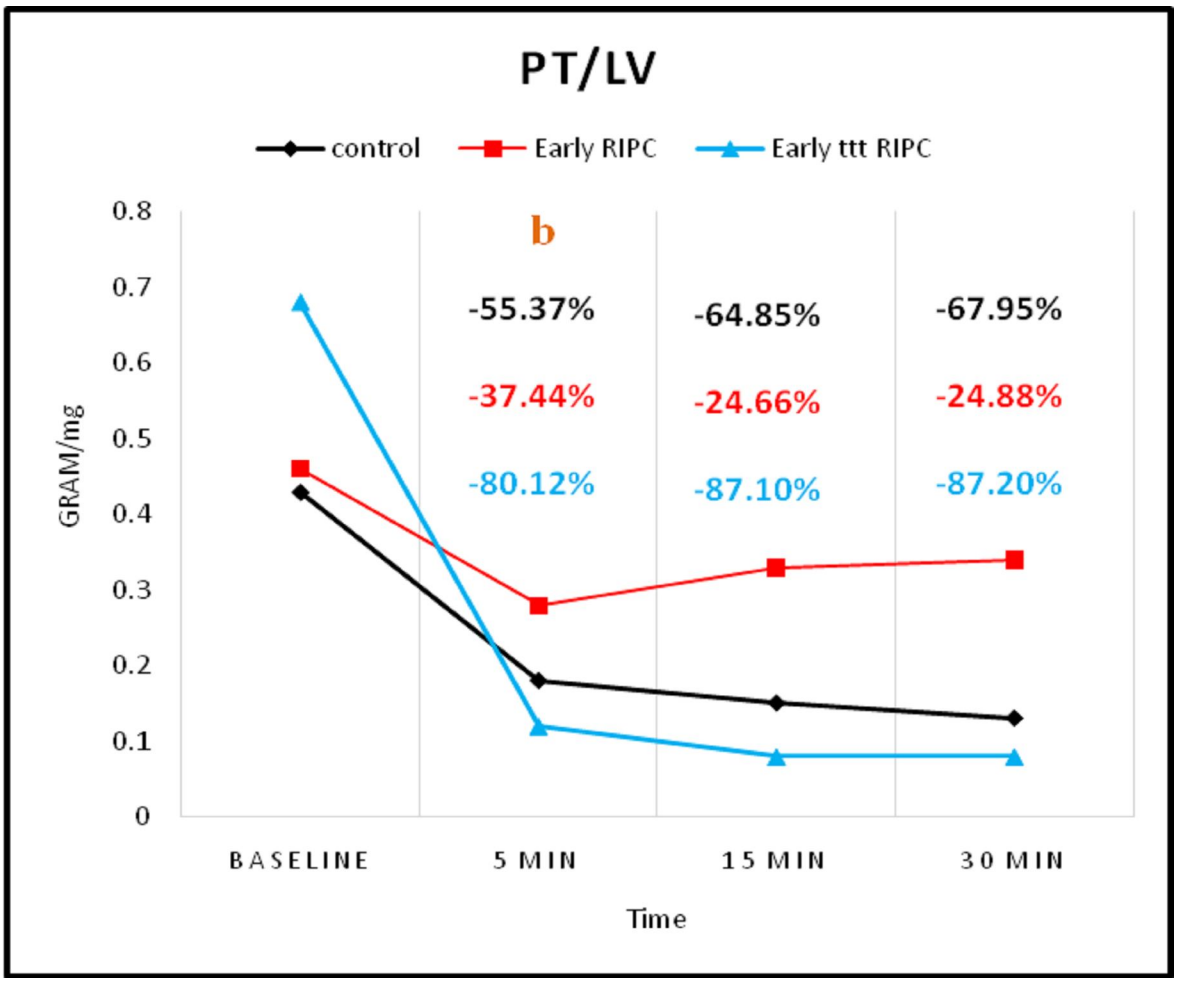


Figure (3): Peak developed tension per left ventricular weight (PT/LV, g/100 mg) preischemia baseline value and responses following ischemia and 30 minutes of reperfusion of hearts isolated from the control, early RIPC and acriflavine treated early RIPC groups.

a: Significance of difference of percent change of non treated group from the control group calculated by LSD at $P < 0.05$.

b: Significance of difference of acriflavine treated early RIPC group from early RIPCgroup calculated by LSD at $P < 0.05$.

The percent of prolongation in TPT significantly decreased in early RIPC group compared to the control group after 15 and 30 min following acriflavine treatment in the early RIPC, the percent of prolongation in TPT significantly increased after 15 and 30 min (Figure 4).

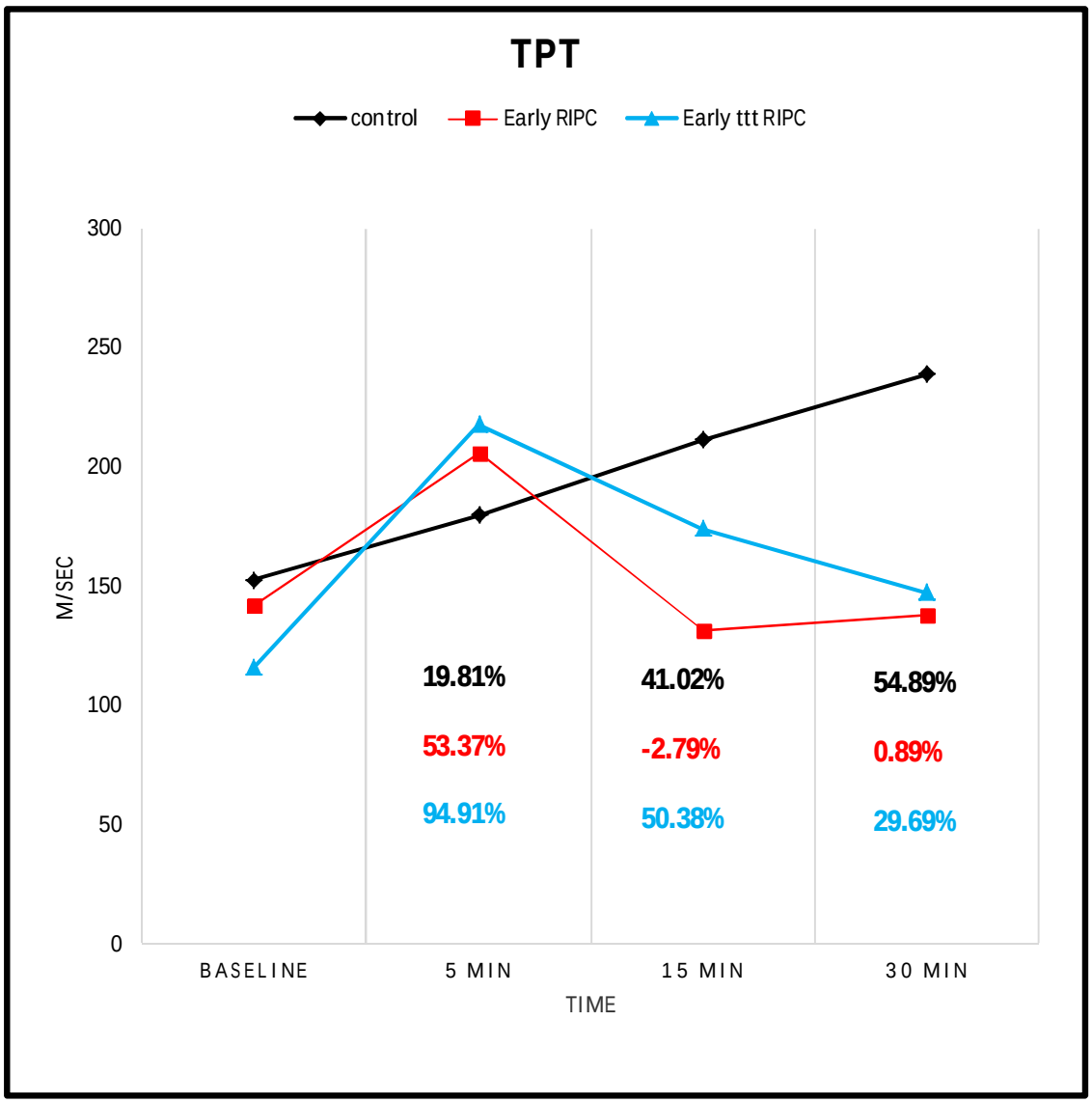


Figure (4): Time to peak tension preischemia baseline value and responses following ischemia and 30 minutes of reperfusion of hearts isolated from the control, early RIPC and acriflavine treated early RIPC groups.

a: Significance of difference of percent change of non treated group from the control group calculated by LSD at $P < 0.05$.

b: Significance of difference of acriflavine treated early RIPC group from early RIPC group calculated by LSD at $P < 0.05$.

The percent of HRT prolongation significantly increased in acriflavine-treated early RIPC group after 5min and at 15 min compared to early RIPC group (Figure 5).

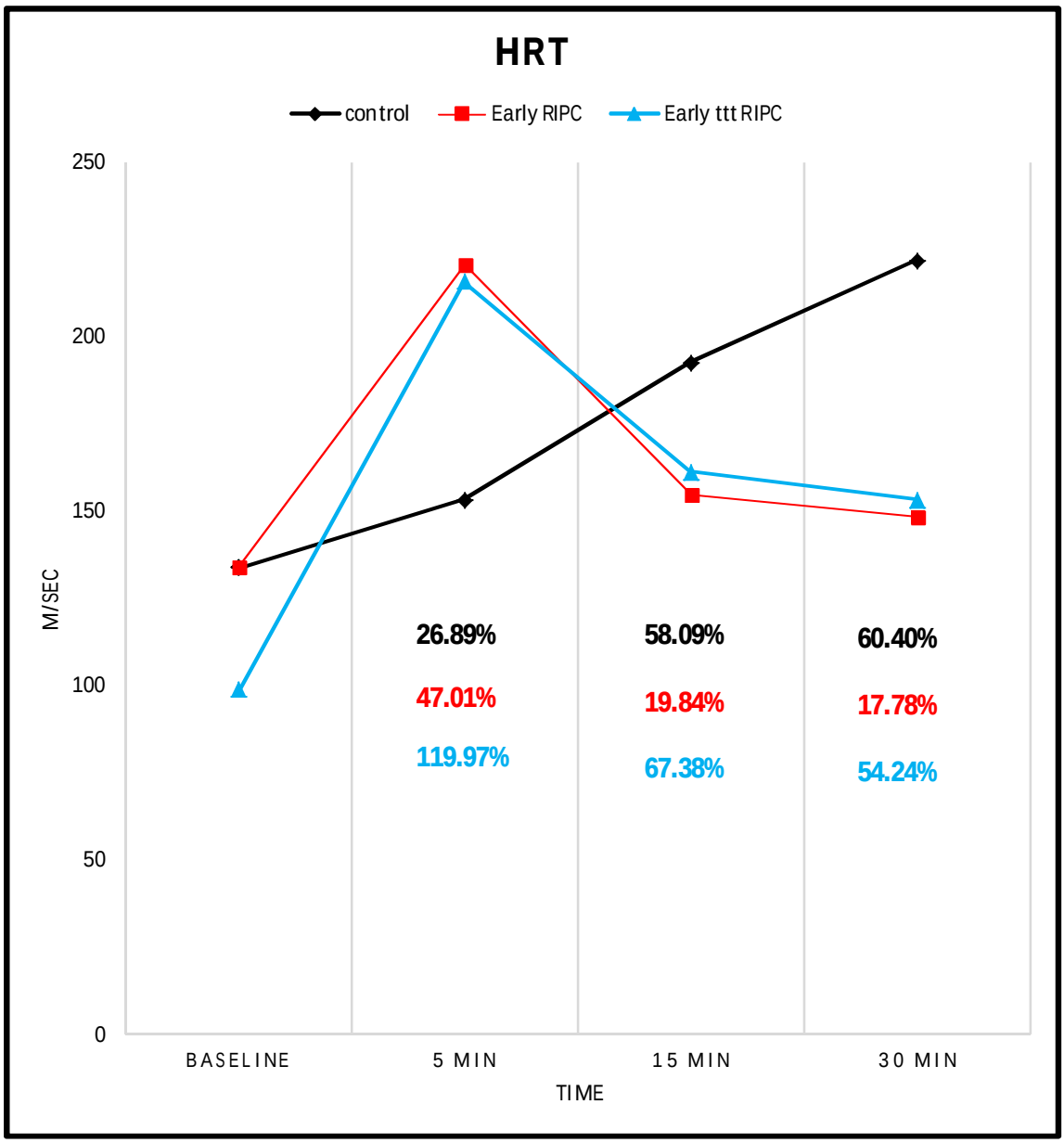


Figure (5): Half relaxation time preischemia baseline value and responses following ischemia and 30 minutes of reperfusion of hearts isolated from the control, early RIPC and acriflavine treated early RIPC groups.

a: Significance of difference of percent change of non treated group from the control group calculated by LSD at $P < 0.05$.

b: Significance of difference of acriflavine treated early RIPC group from early RIPCgroup calculated by LSD at $P < 0.05$.

No significant changes were recorded in MFR or MFR/LV (Figure 6)

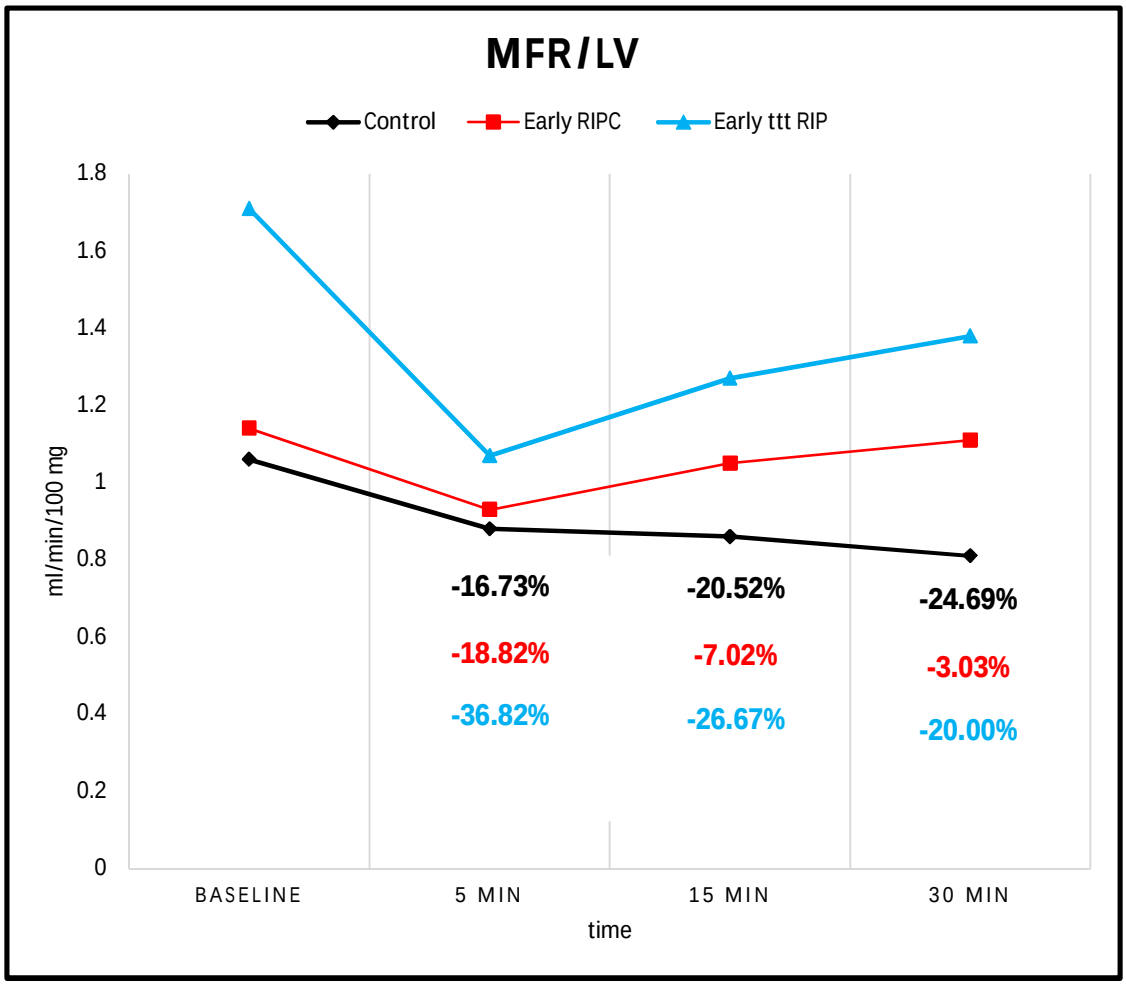
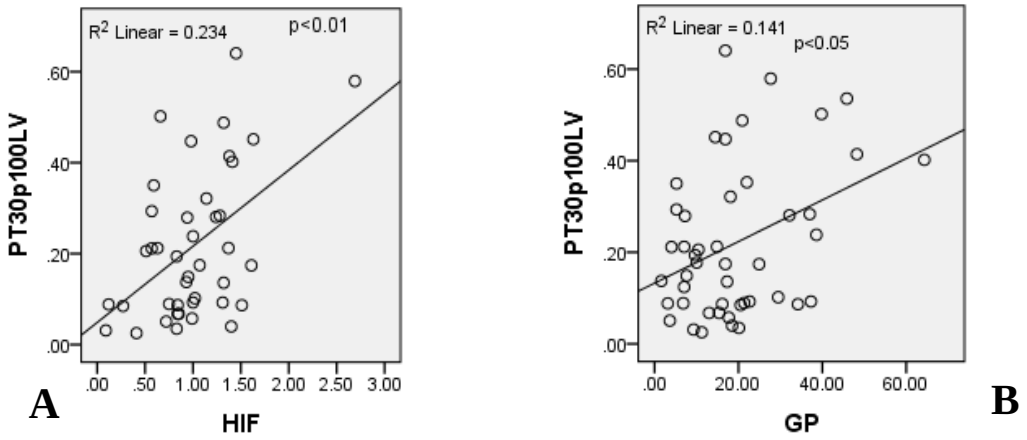


Figure (6): Myocardial flow rate per left ventricular weight (ml/min/100mg) preischemia baseline value and responses following ischemia and 30 minutes of reperfusion of hearts isolated from the control, early RIPC and acriflavine treated early RIPC groups.

a: Significance of difference of percent change of non treated group from the control group calculated by LSD at $P < 0.05$.

b: Significance of difference of acriflavine treated early RIPC group from early RIPCgroup calculated by LSD at $P < 0.05$.

PT/LV was significantly positively correlated to serum HIF level (A) and GPX level (B).



Serum HIF was significantly Negatively correlated to MDA level (C),while it was positively correlated to GPX level (D).

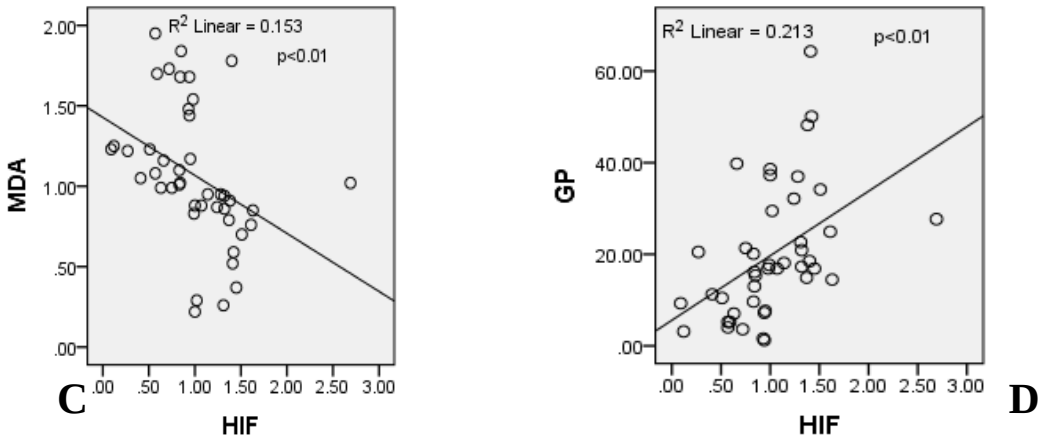


Figure (7): Correlation studies

DISCUSSION

The present study illustrated the effect of remote ischemia preconditioning (RIPC) performed by wrapping a tourniquet around both hind limbs *(Hu et al., 2016 andGhani et al., 2017)* on ischemia reperfusion injury in isolated hearts of rats. Also, the study highlighted the involvement of hypoxia inducible factor in these effects using the HIF-1α inhibitor (Acriflavine), a drug that binds directly to HIF-1 α and HIF-2 α and inhibits HIF-1α dimerization *(Lee et al., 2009)*.

Early RIPC group showed significant increase in the heart rate (HR) and the QTc interval in the ECG records, which can be explained by the in-vivo neural effects of RIPC, as it can activate β adrenergic receptors and enhance sympathetic activity, which was claimed to be mediated by local mediators *(Aimo et al., 2015)*. In-vitro denervation

abolished RIPC beneficial effects on heart rate supporting RIPC neural effects.

In all groups, heart rate, peak developed tension and myocardial flow rate values significantly reduced together with the significant prolongation in time to peak tension and half relaxation time at the end of reperfusion compared to their basal values, but at different degrees and variable percent of changes. These results were consistent with *Elkafoury et al. (2003)*.

The reduction in heart rate following ischemia might reflect depressed automaticity or conductivity with occurrence of various degrees of heart block owing to hypoxia or extracellular potassium excessor may followthe disruption of cardiovascular sympathetic neural responsiveness *(Pomblum et al., 2010)*. This bradycardia might be protective in the context of decreasing cardiac muscle metabolic activity and its oxygen needs which would limit ROS generation *(Barret et al., 2013a)*.

In addition, the significant decrease in PT and PT/LV could be attributed to the decrease in intracellular ATP, and the increase in mitochondrial ROS thus compromising mitochondrial function with lactic acid accumulation and intracellular Ca^{2+} overload which leads to activation of the calcium-activated protease calpain and caspase-3 *(Garcia et al., 2012)*. Also, hypoxia encountered during ischemia induces anerobic metabolism and mitochondrial electron transport chain dysfunction with subsequent ion exchange channel dysfunction , leading to retention of Na^{+}, H^{+}, Ca^{2+} and cell swelling *(Wu et al., 2018)*.

Following reperfusion injury, myocardial stunning, which can be defined as dysfunctional myocardium despite normal or near normal blood flow, ensues leading to post ischemic release of cardio depressant factors and negative inotropic mediators (Pomblum et al., 2010) and to post-ischemic inflammation that can cause cardiotoxicity and disrupt the excitation contraction coupling reducing Ca^{2+} induced Ca^{2+} release *(Canty and Suzuki, 2012)*.

Moreover, oxidative injury with lower activities of antioxidants such as superoxide dismutase and glutathione peroxidase *(Vichova and Motovska, 2013)*, and increased levels of lipid peroxidation end products (MDA) *(Adam, 2014)* could explain deterioration of cardiac functions following I/R injury. *Wu et al. (2018)* stated that, during reperfusion NADPH, NOS and xanthine oxidase systems activate aggregating cell damage and death. ROS directly depresses cardiac contraction and maximal velocity of contraction and relaxation *(Folden et al., 2003)*, inhibits the cell membrane calcium pump, and oxidizes the sarcoplasmic reticulum ryanodine causing intracellular Ca2+overloadand mediates vascular inflammation and atherogenesis *(Cheng et al., 2017)*. Moreover, oxidants can induce cardiac cell apoptosis owing to the disruption of electron transport chain, upregulation of death receptors (Fas) and the loss of mitochondrial membrane pore (MMP) *(Tan et al., 2016)*.

The prolonged TPT or HRT denotes systolic and diastolic dysfunction and so the myocardium takes a longer time to reach the peak of contraction and to become full relaxed, owing to cardiac

remodeling, interstitial edema and fibrosis *(Thune and Solomon, 2006)*.

Post-ischemic endothelial injury with increased endothelin and decreased NO together with neutrophil plugging explain the post-ischemic no-reflow phenomenon and the decrease in the coronary blood flow *(Pomblum et al., 2010).* This may explain the decrease in cardiac function or at least may participate in their occurrence.

Regarding early remote ischemic preconditioning group, it was unable to improve the heart chronotropic activity, which highlights the neural effect of RIPC that is lost following the in-vitro denervation *(Aimo et al., 2015).*

Early RIPC was able to improve cardiac systolic functions evidenced by the significant reduction in the percent of decrease in PT, PT/LV, percent of prolongation in time to peak tension (TPT) and the perfusate lactate dehydrogenase level compared to the control group, with no significant changes in the percent of half relaxation time (HRT) prolongation or in MFR/LV. These results agreed with *Albrecht et al. (2013)* and *Samanta and Dawn (2016)* who detected lower cardiac cellular damage markers troponin I, troponin T, creatinine kinase-MB, and LDH following RIPC, and so assuring its cardioprotection.

In this study, the early RIPC rats were subjected to RIPC 2 hours prior to ischemic /reperfusion injury according to *Tork et al. (2015)*, and this early RIPC phase is activated immediately after preconditioning and vanishes within 4 h and it can mediate cardioprotection by rapid release or posttranslational modification of preexisting proteins *Vanezis et al. (2016)*.

This protective effect could be attributed to their anti-oxidant effects supported in this study by the significant reduction in MDA and elevation in Gpx levels in the RIPC group compared to the control group. *Similarly,Lotfollahi et al. (2016)* suggested that RIPC prevented the enhancement of MDA and increased GPX levels significantly.

Moreover, serum level of HIF 1α was significantly higher in RIPC group than the control group, and was significantly positively correlated to PT/LV, while being negatively correlated to the perfusate LDH enzyme level. Added to this, following acriflavine treatment, the effects of RIPC were abolished highlighting the role of HIF-1-alpha in mediating RIPC protective effects.

Following acriflavine treatment, a significant bradycardia was detected after 5 minutes of reperfusion followed by the occurrence of tachyarrhythmia after 30 min, owing to post ischemic electrical stunning and electrolyte channels dysfunction with the absence of HIF 1α protective effects *(Pomblum et al., 2010)*.

In the acriflavine treated group, a significant increase in the percent of decrease in PT and PT/LV, percent of TPT, HRT prolongation, percent of decrease of MFR and MFR/LV, perfusate lactate dehydrogenase (LDH) and MDA levels were detected together with significantly lower Gpx and HIF 1α levels compared to early RIPC group.

In agreement with these *postulations, Yang et al. (2018)* assured the role of HIF-1 evidenced by the deterioration of the

ischemia/reperfusion insult in rats following acriflavine administration (2 mg/kg/day) for 10 days.

Albrecht et al. (2013) stated that increased HIF-1α following RIPC was accompanied by decrease in the apoptotic and inflammatory cardiac events.

Weber et al. (2015) proved that human plasma retrieved directly after remote ischemic preconditioning (RIPC) was able to reduce hypoxia–induced damage of human endothelial cells cultured in vitro, assuring the release of anti-inflammatory and anti-apoptotic humoral factors into the blood stream after RIPC, from where they reach the remote target organs conferring the RIPC-protection.

Similarly, intraperitoneal injection of HIF activator had a synergistic effect with RIPC on reducing infarction with an increase in IL-4 and IL-10 protein levels in both the peripheral blood and ischemic tissue, while the injection of the HIF inhibitor (acriflavine hydrochloride) abolished the protective effects of RIPC on infarction, and reduced IL-4 and IL-10 protein levels in both the peripheral blood and ischemic tissue *(Yang et al., 2018).*

HIF-1 α was proved to have an anti-apoptotic role by increasing MCL-1 expression, a Bcl-2 related anti-apoptotic gene, and by suppressing cytochrome C release from mitochondria via heterodimerization and neutralization of pro-apoptotic proteins such as Bim or Bax *(Flamant et al., 2010)*. Moreover, HIF-1 α also can inhibit mitochondrial oxidative metabolism, thereby reducing the generation of reactive oxygen species under conditions of hypoxia or hypoxia-reoxygenation *(Semenza, 2014)*.

This was supported in our study by the positive correlation between the HIF-1 α and the cardiac tissue antioxidant capacity (Gpx), while being negatively correlated to the cardiac tissue (MDA).

Finally, on the contrary, some studies failed to show any significant difference between RIPC and control groups, regarding the level of troponin release *(Meybohm et al., 2015).* Also, *Hausenloy et al. (2015)* reportedno improvement in CABG patients that had RIPC. Both *Candilio & Hausenloy (2017)* and *Pierce et al. (2017)* denied any clinical benefit from RIPC procedure suggesting that propofol (used during anesthesia) may interact with the protective effects of RIPC pointing to the neural factors that can also mediate RIPC effects.

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الدور المحتمل للتهيئة المبكرة بعيدة المدي التي تسبب في حماية القلب ضد الاصابة الناتجة عن الإقفار الدموي بالقلوب المعزولة للجرذان وإحتمالية وجود دور للعامل المحفز بنقص الأكسجين

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خلفية البحث : تعتبرأمراض القلب والشرايين مساهما رئيسيا في معظم الحالات المرضية والوفيات في العالم و تمثل حوالي ثلث أو أكثر من مجموع الوفيات.وتمثل التهيئة الموضعية التى تسبق الإقفارالدموى تقنية تجريبية تتعرض فيها الانسجة لنوبات قصيرة من نقص التروية لتحميها من الخلل الناتج عن قطع التغذية وإعادة سريانها.

الهدف من البحث: تهدف هذه الدراسة إلى تقييم دورالتهيئة بعيدة المدى التى تسبق الإقفارالدموى على الحد من إصابة عضلة القلب في الجرذان نتيجة نقص التروية و إعادة ضخها،ولإلقاء الضوء على الدور الذي يمكن أن يقوم به العامل المحفز لنقص الأكسجبين ١ الفا في هذه الحماية.

مواد وطرق البحث: تم أجراء هذا البحث على٢٨ جرذا من الجرذان البالغة. وتم إيواء الحيوانات فىMSRI مركز الابحاث في ، كلية طب، جامعة عين شمس تحت الظروف المناسبة من الضوء و التهوية و درجة الحرارة(٢٢-٢٥ درجة مئوية) وحرية الوصول إلى الغذاء و الماء. و تم وضع الجرذان فى البيئة الجديدة لمدة ٧ أيام قبل إجراء التجربة و تقسيمهم عشوائيا إلى المجموعات الاتية:

أ- **المجموعة الضابطة :** تضم الجرذان التي تعرضت قلوبها لنقص التروية وإعادة سريانها فقط.

ب- **المجموعة المعالجة بالتهيئة المبكرة بعيدة المدي التي تسبق الأقفار:** تضم الجرذان التي تتعرضت للتهيئة بعيدة المديساعتين قبل تعرض قلوبها المعزولة لنقص التروية وإعادة سريانها .

ج- **المجموعة المعالجة بالتهيئة المبكرة بعيدة المدي التي تسبق الأقفار و بعقار الأكريفلافين:** تضم الجرذان التي عرضت للحقن بالأكريفلافين لمدة ١٠ أيام قبل تعرضها للتهيئة بعيدة المدى التي تسبق تعرض القلوب المعزولة لنقص التروية وإعادة سريانها بساعتين.

وقد تم عمل القياسات الآتية:

١. قياس وزن الجسم.

٢. تسجيل رسم القلب.

٣. قياس أداء القلوب المعزولة على جهاز لانجندورف من حيث الوظائف الأساسية لعضلة القلب و خلال ٣٠ دقيقة من إعادة التروية بعد ٣٠ دقيقة من الإقفار الدموى.

٤. قياس دلائل الأكسدة في أنسجة القلب.

٥. أخذ عينات من سائل الارواء لتقييم نسبة.LDH

٦. قياس مستوىالعامل المحفز لنقص الأكسجبين ١ ألفافى المصل.

النتائج: أظهرت النتائج ان مجموعة التهيئة المبكرة بعيدة المدي التي تسبق الإفقارRIPC)) الدموى كانت قادرة علي تحسين قوة إنقباض القلب ،ويستدل علي ذلك بإنخفاض ملحوظ في نسبة إنخفاض PT,PT/LV في ١٥و٣٠ د قيقة من إعادة سريان الدم،مع إنخفاض نسبة الوقت المتخذ للوصول لقمة إنقباض القلبو إنخفاضمستوي انزيم Lactate dehydrogenase بشكل ملحوظ مقارنة بالمجموعة الضابطة. ويمكن أن يعزي هذا التأثير الوقائي الي آثارها المضادة للأكسدة المدعومة في هذه الدراسة من خلال إنخفاض كبير في Malonaldehyde،وإرتفاع في مستوي Glutathione peroxidase.

وعلاوة علي ذلك،كان مستوي العامل المحفز بنقص الأكسجينأعلي بكثيرمقارنة بالمجموعة الضابطةمع إرتباطه بشكل ايجابي مع قوة الانقباض في البطين الأيسر،بينما يرتبط إرتباطا سلبيا بمستويإنزيمLactate dehydrogenaseفي سائل الإرواء.

مما يبرزدور ال HIF-1α في التوسط للتأثيرات الوقائية للتهيئة المبكرة بعيدة المدي التي تسبق الإقفار. وقد أثبت هذا المفهوم من خلال إعطاء علاج الأكريفلافين وهو أحد المثبطات الأكثر فاعلية للHIF-1α حيث إنخفض مستوي الHIF-1αبشكل ملحوظ في المجموعة المعالجة بالأكريفلافين مقارنة مع المجموعة الغير معالجة بالأكريفلافين والمجموعة الضابطة ، مع إختفاء النتائج الايجابية للتهيئة المبكرة بعيدة المديوقد ترافقت هذه النتائج مع إرتفاع Malonaldehyde وإنخفاض في مستوي Glutathione peroxidase مقارنة مع مجموعاتالتهيئة المبكرة بعيدة المدي التي تسبق الإقفار.

الاستنتاج: التهيئة المبكرة بعيدة المدي التي تسبق الإقفار تقنية غير مسبوقة وغير دوائية ويمكن أن تساعد في إصلاح إصابة نقص التروية وإعادة سريانها ،مع ظهور دور واضحللعامل المحفز بنقص الأكسجينفي التوسط في تلك الوظائف الوقائية.