ROLE OF VISFATIN IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY EXPERIMENTALLY INDUCED IN ADULT MALE ALBINO RATS

Salah I. Zaghloul, Azza A. Megahed, Randa S. Gomaa and Eman A. Mohamed
Physiology Department, faculty of medicine, Zagazig University

ABSTRACT

Background: Visfatin is an adipocytokine capable of mimicking the glucose-lowering effects of insulin. Whether visfatin is capable of exerting cardioprotective effect is still uncler. **Objective:** The goal of this study was to demonstrate the role of visfatin in myocardial ischemia/reperfusion injury in adult male albino rats. **Design:** Total number of forty healthy, adult, male albino rats were divided into four equal groups (n=10) Group 1 (I) : the rats were subjected to ischemia for 30 min, Group 2 (I/R): the rats were subjected to ischemia for 30 min and 120 min reperfusion, Group 3 (I/R+V): the rats were subjected to ischemia and reperfusion and visfatin was injected immediately prior reperfusion and Group 4 (I/R+V30): the rats were subjected to ischemia and reperfusion and visfatin was injected after 30 min of reperfusion. Mean arterial blood pressure (MABP) and heart rate (HR) were measured at 0 min and 15 min. into ischemia, and at 5, 30 and 120 min. into reperfusion. At the end of the experiment infarct size was determined by tetrazolium staining (TTC), serum level of lactate dehydrogenase enzyme LDH, creatine kinase –MB enzyme CK-MB, and malondialdehyde MDA were calculated. **Results:** Injection of visfatin either immediately prior reperfusion or after 30 min of reperfusion produced insignificant changes in MABP and HR when compared by rats subjected to ischemia reperfusion injury. There was a significant increase in infraction size, LDH, CK-MB and MDA levels in (I/R) group and (I/R+V30) group in comparison with (I) group but there was insignificant change in all the mentioned parameters in (I/R+V) group when compared with (I) group. There was a significant decrease in infraction size, LDH, CK-MB and MDA levels in (I/R+V) group compared with (I/R) group however there was insignificant change in all parameters in (I/R+V30) group when compared with (I/R) group. **Conclusion:** Acute administration of exogenous visfatin can protect against acute myocardial ischaemia-reperfusion injury in a non-atherosclerotic animal model of myocardial infarction. This protection is due to direct reducing effect on infarction size that may be attributed to reduction in oxidative stress process as indicated by the decrease in MDA level.

Key words: visfatin, ischemia/reperfusion injury.

INTRODUCTION

Visceral fat accumulation, a key feature of the metabolic syndrome ‹is associated with the development of diabetes mellitus, a three-fold increased risk of developing coronary heart disease [(1)] two to three times increase in cardiovascular mortality [(2)] and worse clinical outcome following an acute myocardial infarction or a primary percutaneous coronary intervention [(3)].

Although formerly regarded as purely an energy storage site, the emerging studies suggest that adipose tissue is an active endocrine organ producing ‘adipocytokines’, hormones that influence a diverse array of processes including appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and haemostasis , all factors that can impact cardiovascular disease [(4)]. In this regard, the adipocytokines, adiponectin, leptin and apelin, have been linked to cardioprotection in recent experimental studies [(5)].

The recently discovered adipocytokine, visfatin, has also been identified as the enzyme nicotinamide phosphoribosyl transferase (Nampt), visfatin was formerly identified as a pre-B cell colony-enhancing factor (PBEF) and has been demonstrated to mimic the glucose-lowering effect of insulin and improve insulin sensitivity [(6)].

Furthermore, by binding to the insulin receptor, visfatin has been demonstrated to activate intracellular kinase signalling cascades, such as the phosphatidylinositol 3-kinase-Akt) (PI3-K-Akt) and mitogen- activated protein kinase (MAPK) pathways, through which it may exert an antiapoptotic effect [(7)].

Reperfusion of the previously-ischemic myocardium is often followed by the detrimental changes in coronary arteries and myocardial tissues, which ultimately results in cardiac dysfunction, known as ischemia/reperfusion (I/R) injury. I/R injury has been implicated in the pathology of peripheral vascular insufficiency, angina, myocardial infarction and stroke[(8)].

Visfatin has the ability to activate Akt and MAPK, these are kinases belong to the reperfusion injury salvage kinase (RISK) pathway [(9)] a group of protein kinases that on activation at the time of myocardial reperfusion confer powerful cardioprotection, an effect attributable, in part, to the inhibition of the mitochondrial permeability transition pore (m PTP) [(10)]. The mPTP is a non-specific mitochondrial channel whose opening in the first few minutes of myocardial reperfusion is a critical determinant of the cardiomyocyte death [(11)].

However, ***Romacho et al., (2009)***[(12)] reported that Visfatin/PBEF/Nampt as an adipocytokine that can promote vascular smooth muscle cell

inflammation, this effect is not mediated by the activation of the insulin receptor, but rather relies on the intrinsic Nampt activity .visfatin may have a direct role in vascular dysfunction and may be involved in the pathogenesis of myocardial fibrosis in patients with coronary artery disease (CAD) and acute myocardial infarction following ischemia/reperfusion (I/R) injury**.**

The goal of this study was to demonstrate the role of visfatin on myocardial ischemia/reperfusion injury in adult male albino rats.

MATERIAL AND METHODS

Animals:

A total number of forty healthy, male albino rats weighing 180- 220 gm were used. All the animals were bred in the animal house. Animals had free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. The rats were accommodated to animal house conditions for two weeks before the experiments going on. Experiments were performed between 9:00 AM and 5:00 PM.

Animal preparation and instrumentation:

The rat was anesthetized with urethane (ethyl carbamate) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) 25% freshly prepared solution in a dose of 1.25- 1.5 gm/kg injected intrapertioneally [(13)].

A suitable distance tracheotomy was done through which the rats were intubated and ventilated with a ventilator (Animal pump, miniature ideal pump assembly 230V; Bioscience UK)

The external jugular vein and carotid artery were isolated and cannulated for drug administration and mean arterial blood pressure (MABP) measurement (PT400 blood pressure transducer), respectively.

FC123 ECG limb cable was attached through hypodermic needles inserted and fixed subcutaneously and it was switched on lead II that gives good analysis of the ECG and calculation of the heart rate /minute. After stabilization of the blood pressure and heart rate for 15 minutes, experiment was performed.

In vivo infarct induction:

A left thoracotomy was performed, between the 4th and the 5th intercostal spaces. The heart was exteriorized through lateral compression of the chest and the left anterior descending (LAD) coronary artery was ligated with 6-0 silk suture thread at approximately 2 mm from its origin, between the left atrial border and the pulmonary artery sulcus. Then, the heart was rapidly returned to the thoracic cavity, the lungs were expanded with positive ventilation with 100% oxygen. Hearts were subjected to 30 min regional ischemia before hearts were reperfused for 120 min [(14)]. Myocardial reperfusion was initiated by release of the silk suture. During coronary artery ligation and reperfusion the thoracic cavity was covered with a sterile saline solution saturated swab to prevent excess fluid loss and dehydration. Rats were also injected with 2ml of sterile saline intraperitonealy every 30 minutes to compensate for any fluid loss which may have occurred due to the ventilation. Successful LAD coronary artery occlusion was confirmed by the presence of ST elevation and a decrease in the arterial blood pressure ***(Tracing 1)***.

Experimental protocol:

The animals were randomly divided into 4 equal groups and assigned to one of the following experimental protocols ***(Fig. 1):***

Group 1 (I) (n=10)**:** In which the rats were subjected to ischemia for 30 minutes.

Group 2 (I/R) (n=10): In which the rats were subjected to ischemia for 30 minutes and 120 min reperfusion.

Group 3 (I/R+V) (n=10): In which the rats were subjected to ischemia for 30 minutes and 120 min reperfusion and visfatin was injected immediately prior reperfusion.

Group 4 (I/R+V30) (n=10)**:** In which the rats were subjected to ischemia for 30 minutes and 120 min reperfusion and visfatin was injected after 30 min of reperfusion.

Visfatin (Sigma-Aldrich Chemical, St. Louis, MO) was given as an intravenous bolus (5×10^{-6} µmol, 0.1 ml) dissolved in normal saline [(15)].

Assessment of cardiovascular parameters:

Mean arterial blood pressure (MABP) and heart rate (HR) were measured at 0 and 15 min. into occlusion (I, 0 min. and I, 15 min.), and at 5, 30 and 120 min. into reperfusion (R, 5 min.; R, 30 min. and R, 120 min.).

Assessment of myocardial injury:

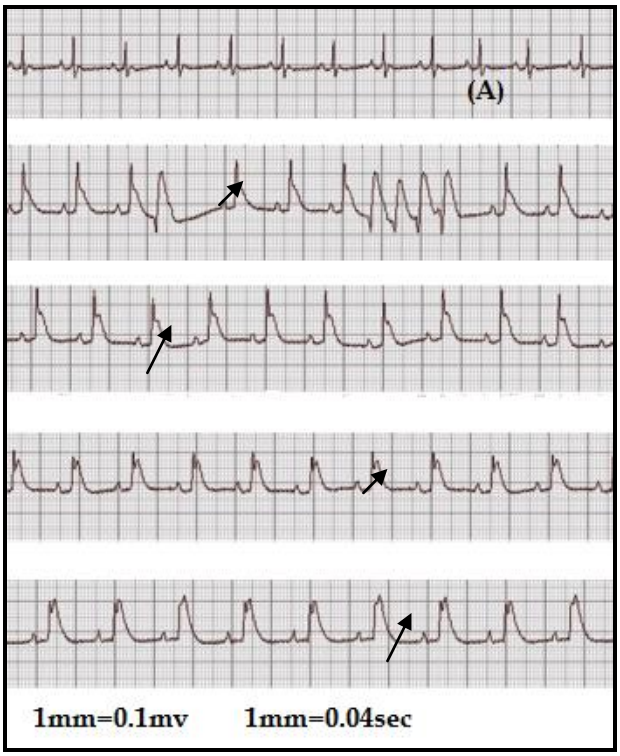
Infraction size was measured by the tetrazolium technique (triphenyl tetrazolium choloride stain (TTC): Bio Basic INC, USA) ***(fig 2)***. At the end of the experiments, each heart was rapidly isolated and following 20 min of incubation at 37°C in 0.1% solution of tetrazolium in phosphate buffer (8.0 gm/L NaCL, 0.2 gm/L Kcl, 1.44gm/L Na_2Hpo_4 and 0.24 KH_2po_4 gm/L), stained viable tissue was carefully separated from unstained necrotic tissue and then weighed. The necrotic mass was expressed as a percentage of the total left ventricular mass [(16)]**.**

At the end of each experiment blood samples were obtained from sinus orbitus vein. The blood samples were allowed to clot at room temperature before centrifuging for 20 minutes at

approximately 7000 rotation /min. The serum was separated and stored at -20° C in a dark container until measurement of: lactate dehydrogenase enzyme (LDH) using (LDH Kits; Vitro Scient, Egypt), creatine kinase –MB enzyme (CK-MB) using (CK-MB Kits; Bio-diagnostic, Egypt) and malondialdehyde (MDA) using (MDA Kits; Bio-diagnostic, Egypt) as the determination of (MDA) is one of the most commonly used methods for monitoring lipid peroxidation and oxidative stress reaction [17].

Statistical Analysis:

The data were expressed as mean ± SD for quantitative variables and statistically analyzed according to the methods described by ***Kirkwood (1989)*** [18]**.** The statistical analysis was done by using SPSS program (version 17) (SPSS Inc. Chicago, IL, USA). Multiple comparisons were made by one-way analysis of variance **(ANOVA).** Subsequent post hoc analysis to determine significant differences between two groups were performed by least significant difference (LSD) test. Test was considered significant at P values < 0.05.



Tracing 1: ECG recordings of normal and ischemic rats. (A) Normal ECG shows normal ST segment. The arrow indicates ST segment elevation ischemia

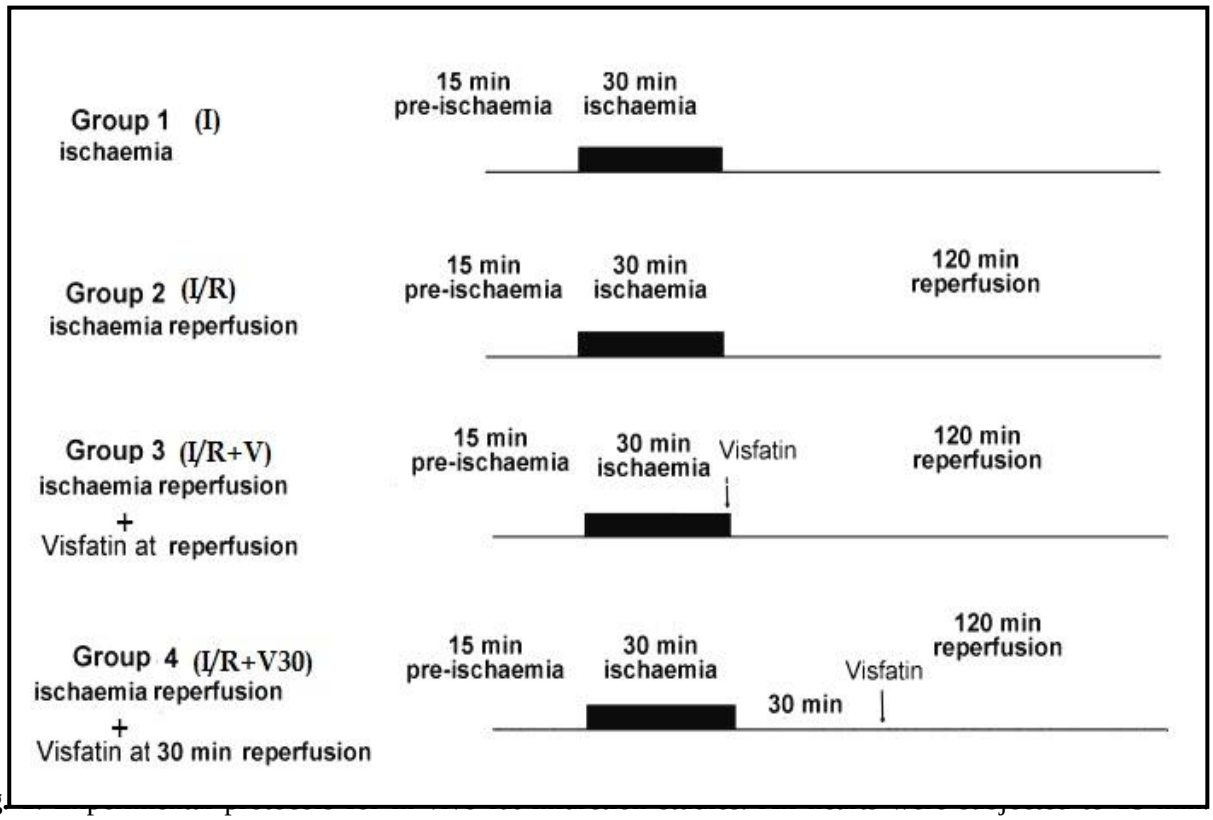


Fig. of stabilization; 30 min. of regional myocardial ischemia, followed by 120 min. of myocardial reperfusion except group 1. Visfatin was given immediately prior to the onset of myocardial reperfusion in group 3 and 30 min. after reperfusion in group 4.

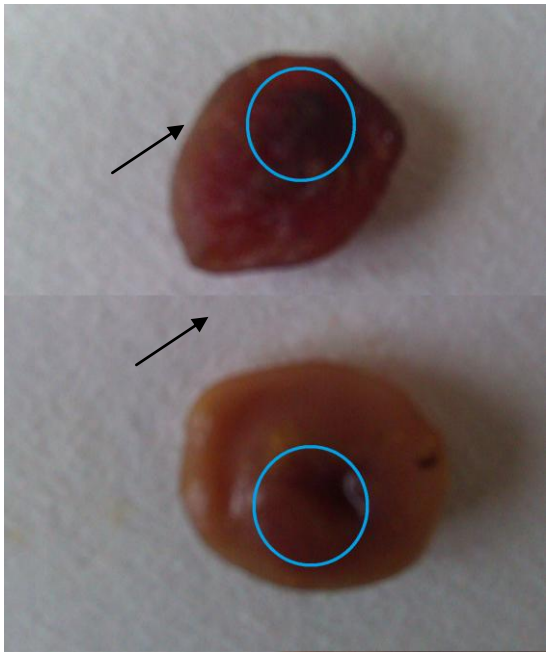


Fig. 2: Normal myocardium is stainted by TTC to a red color (pointed by arrow) and lack of staining of acutely infracted tissue (encircled parts in the figure).

RESULTS

Cardiovascular parameters

Table (1) shows cardiovascular parameters; Mean arterial blood pressure (MABP) and heart rate (HR) recorded in the four studied groups at base line (0 min) and 15 min. into occlusion (I, 0 min. and I, 15 min.), and at 5, 30 and 120 min. into reperfusion (R, 5 min.; R, 30 min. and R, 120 min.). At baseline (I, 0 min)and (I, 15 min) both MABP and HR showed insignificant changes

among all studied groups (P>0.05). Injection of visfatin either immediately prior reperfusion or after 30 min of reperfusion produced insignificant changes in MABP and HR when compared by rats subjected to ischemia reperfusion injury at R, 5 min.; R, 30 min. and R, 120 min (P>0.05).

Myocardial injury parameters:

Table 2, Fig. 3A show that there was a significant increase in infraction size, LDH, CK-MB and MDA levels in ischemia/reperfusion group and ischemia/reperfusion + visfatin injection after 30 min of reperfusion group in comparison with ischemic group (P<0.001) but there was insignificant change in all the mentioned parameters in ischemia/reperfusion + visfatin injection immediately prior reperfusion group when compared with ischemic group (P>0.05). There was a significant decrease in infraction size, LDH, CK-MB and MDA levels in ischemia/reperfusion + visfatin injection immediately prior reperfusion group compared with ischemia/reperfusion group (P<0.001) however there was insignificant change in all parameters in ischemia/reperfusion + visfatin injection after 30 min of reperfusion group when compared with ischemia/reperfusion group (P>0.05).

Table (1): Myocardial performance parameters in all studied groups:

Groups	Mean Arterial Bl P (MABP) (mmHg) Time-point				
	I, 0 min	**I, 15 min**	**R, 5 min**	**R, 30 min.**	**R, 120 min**
(I)	119.3±6.7	95.7±3.4	———	———	———
(I/R)	120.7±5.1	93.9±6.1	86.5±8.1	80.2±4.3	57.2±5.4
(I/R+V)	122.1±5.3	96.3±5.4	85.7±5.2	79.1±2.9	62.1±6.3
(I/R+V30)	115.8±4.6	94.2±4.7	83.6±2.9	77.3±4.8	58.9±3.2
Groups	Heart Rate (HR) (beats/min) Time-point				
	I, 0 min	**I, 15 min**	**R, 5 min**	**R, 30 min.**	**R, 120 min**
(I)	335.3±56.3	320.6±54.5	———	———	———
(I/R)	332.1±65.8	328.2±46.3	312.5±58.3	304.5±64.7	307.3±45.8
(I/R+V)	334.9±54.1	330.7±65.4	321.7±55.3	316.4±42.5	301.7±53.4
(I/R+V30)	331.3±47.4	323.4±58.2	325.6±61.1	311.6±54.3	229.5±56.2

Table (2): Myocardial ischemia-reperfusion injury: Infarct size, lactate dehydrogenase(LDH), creatine kinase –MB enzyme (CK-MB) and malondialdehyde (MDA) in all studied groups

Groups	Infarction size (% of LV mass)	LDH (IU/L)	CK-MB (IU/L)	MDA (nmol/ml)
(I)	39.9±3.4	613.5±57.3	249.6±36.1	1.7±0.87
(I/R)	46.9±2.3***	858.9±48.4***	416±52.5***	5.8±1.1***
(I/R+V)	37.5±2.8###	632.3±63.7###	278.5±36.5###	2.3±0.73###
(I/R+V30)	47.3±2.8***	861±53.9***	463.1±31.8***	6.1±1.06***

***significant when compared with (I) group (P<0.001).

significant when compared with (I/R) group (P<0.001)

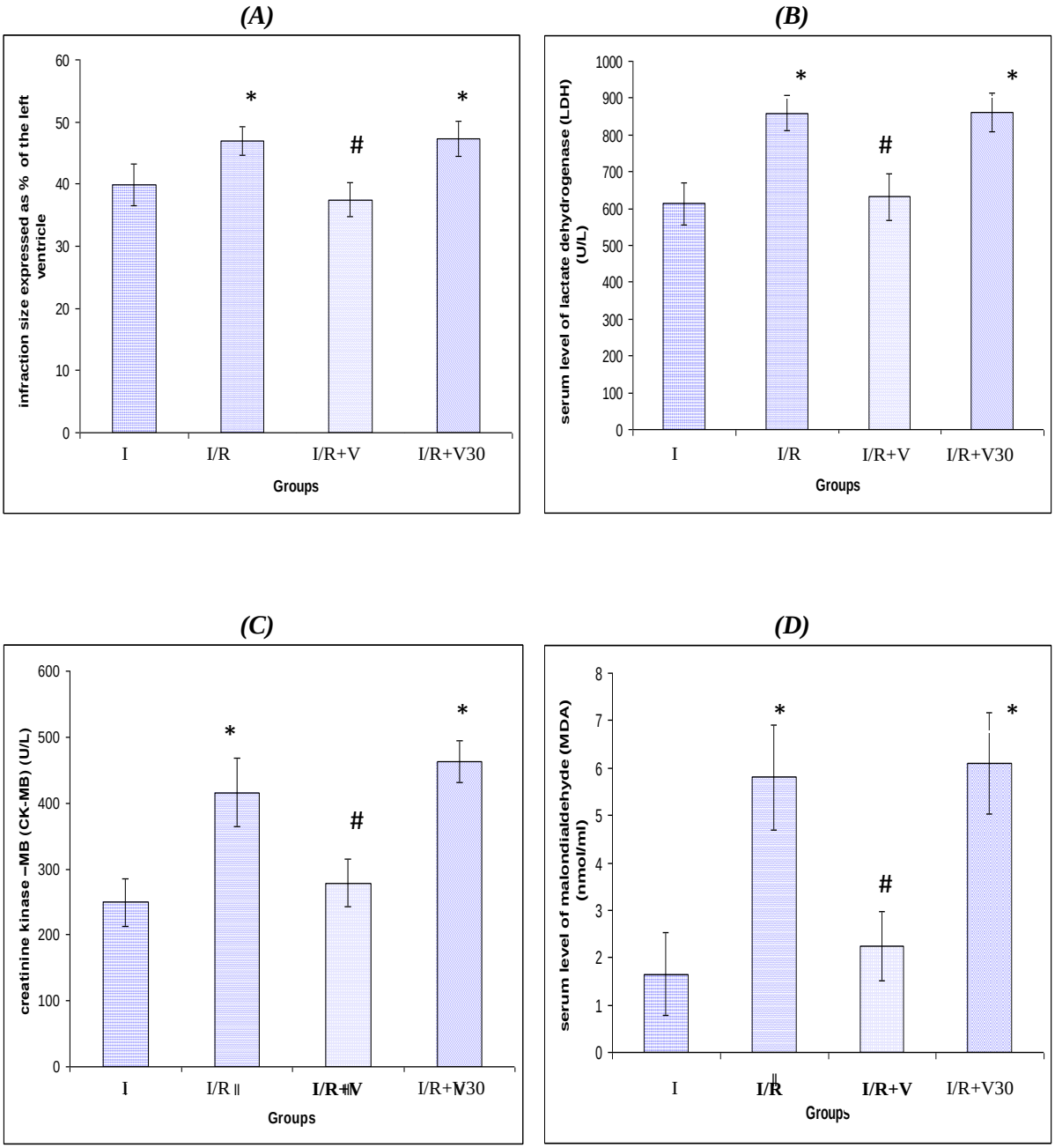


Figure (3): Myocardial ischemia-reperfusion injury. (A): Infarct size. (B): lactate dehydrogenase(LDH) release. (C): creatine kinase –MB enzyme (CK-MB) release. (D) malondialdehyde (MDA) release

DISCUSSION

The present study showed that at baseline (I, 0 min) and (I, 15 min) both MABP and HR showed insignificant changes among all studied groups and injection of visfatin either immediately prior reperfusion or after 30 min of reperfusion produced insignificant changes in MABP and HR when compared by rats subjected to ischemia reperfusion injury at R, 5 min.; R, 30 min. and R, 120 min.

These results are similer to that obtained by ***Lim et al. (2008)*** [15] who studied the cardio protective effect of visfatin on C57BL/6 male mice.

The present study showed a significant increase in infraction size, LDH, CK-MB and MDA levels in ischemia/reperfusion group and ischemia/reperfusion + visfatin injection after 30 min of reperfusion group in comparison with ischemic group but there was insignificant change in these parameters in ischemia/reperfusion + visfatin injection immediately prior reperfusion group when compared with ischemic group. There was a significant decrease in infraction size, LDH, CK-MB and MDA levels in ischemia/reperfusion + visfatin injection immediately prior reperfusion group compared with ischemia/reperfusion group however there was insignificant change in all parameters in ischemia/reperfusion + visfatin injection after 30 min of reperfusion group when compared with ischemia/reperfusion group.

These results are in agreement with ***Lim et al. (2008)*** [15] who used an in vivo murine infarction model and found that administration visfatin, specifically at the time of myocardial reperfusion, dramatically reduce the myocardial infarct size. They attributed observations that visfatin could delay the opening of the m PTP in isolated cardiomyocytes subjected to oxidative stress.

Smith and Yellon (2011) [19] found that visfatin protects against I/R injury by activation of kinases which are key elements of the mechanisms underlying tissue preservation, including the Reperfusion Injury Salvage Kinase (RISK) pathway components PI3K-Akt (phosphatidylinositol 3-OH kinase-cellular Akt/protein kinase B) and p44/42 (mitogen-activated protein kinase), and inhibition of the mitochondrial permeability transition pore (m PTP).

Adding support to the findings of the present study, **Chiao-Po et al. (2009)**[20] showed that Nampt expression in the heart significantly reduced the size of myocardial infarction after ischemia and I/R, suggesting that Nampt has a protective function in the heart in vivo. They found that expression of Nampt (visfatin) in the heart is down regulated in response to ischemia and down regulation of visfatin induces cell death, so supplementing visfatin, may be considered as a novel cardiovascular treatment because visfatin plays an important role in mediating life-span extension through accelerating clearance of damaged mitochondria by maintaining mitochondria in a healthy state and reducing oxidative stress.

Visfatin may not only provide a potential new target for acute cardioprotection but it may also act as an anti-diabetic agent with a unique mechanism of action, thereby offering a potentially novel drug target for the diabetic patient that experiences an episode of acute myocardial ischaemia-reperfusion injury [21].

In contrast with these results ***Bae et al., (2006)***[22] reported that in the experimental studies the visfatin gene is induced in response to hypoxia, an effect mediated by hypoxia inducible factor, raising the possibility that visfatin may be up-regulated in response to myocardial ischaemia and responsible for myocardial damage.

In addition to, **Yang et al. (2009)**[23] found that increased serum visfatin levels are associated to coronary artery disease (CAD) and acute coronary syndromes, it is well established that acute ischemic stroke is another possible consequence of atherosclerosis. Recently, higher visfatin levels were found in Chinese individuals with stroke, suggesting a possible role in this vascular disease.

Also, ***Yu et al., (2011)*** [24] reported that increased plasma visfatin concentrations in patients with acute ST-Segment Elevation Myocardial Infarction (STEMI). They suggest that visfatin may contribute to post infarction damage dependent on myocardial kinases reduction. They found that the visfatin levels were positively associated with cardiac enzymes. Thus, visfatin level may be closely related to myocardial damage as a higher level of myocardial iso-enzyme means more myocardial damage and a larger infarction area.

In addition ***Lu et al., (2012)*** [25] found that Plasma levels of visfatin were significantly increased in patients with STEMI relative to controls (effort angina patients and individuals without coronary artery disease). The visfatin levels reached a peak 24h after percutaneous coronary intervention (PCI) and then decreased toward the control range during the first week after PCI. The basal plasma visfatin levels were found to correlate with peak troponin-I, peak creatine kinase-MB and total white blood cell count levels.

Further, in the same study they noticed that in myocardial infarction mice, mRNA levels of visfatin were found to be higher than in sham-treated mice. This study indicates that plasma visfatin levels are significantly higher in STEMI patients and that these higher visfatin levels correlate with elevated levels of cardiac enzymes, suggesting that increased plasma visfatin may be closely related to the degree of myocardial damage[25].

In the current study, it was found that the acute administration of exogenous visfatin can protect against acute myocardial ischaemia-reperfusion injury in a non-atherosclerotic animal model of myocardial infarction. This protection is due to direct reducing effect on infarction size that may be attributed to reduction in oxidative stress process as indicated by the decrease in MDA level, which could be a key to open the door for further studies to clarify the underlying mechanisms of this protective effect.

Therefore, further studies are recommended on the pharmacological cardiovascular actions of visfatin that might represent a novel therapeutic approach to prevent and treat cardio-metabolic complications.

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دورالفيسفاتين في نقص الإمداد الدموي وإعادة الإرواء المحدث تجريبياً في عضلة القلب فى ذكور الجرذان البالغة

من المسلم به الآن أن الخلايا الدهنية تعتبر من الغدد الصماء التي تفرز العديد من المركبات التي تختص بمجموعة واسعة من الوظائف وتسمي هذه المركبات نسبة الي الخلايا الدهنية أديبوكينات. و من المعلوم أن تلف عضلة القلب الناتج عن نقص الإمداد الدموي وإعادة الإرواء يعتبر من الصور الشائعة في أمراض القلب ونتيجة لبعض أنواع العلاجات مثل إذابة الجلطة- قسطرة القلب- زراعة القلب .....إلخ. يمكن الحصول علي حماية ملحوظة ضد نقص الإمداد الدموي و إعادة الإرواء إما قبل الامداد الدموي بفترة وجيزة أو عند إعادة الإرواء من خلال تنشيط مسارات محددة. و قد تم تسليط الضوء في الدراسات السابقة علي الفيسفاتين كعامل وقائي ضد تلف عضلة القلب أثناء إعادة الإرواء و لأن البيانات حول هذا الموضوع متضاربة فقد تم تصميم هذه الدراسة لتقييم دور الفيسفاتين في حمايه عضله القلب من تاثير نقص الإمداد الدموي وإعاده الإرواء ومحاولة تحديد آلية هذا الدور.

في هذه الدراسة خضعت الجرذان لنقص الإمداد الدموي وإعاده الإرواء الذي يتألف من ٣٠ دقيقة من نقص الإمداد الدموي تليها ١٢٠ دقيقة من إعاده الإرواء. وقد تم تعيين هذه الجرذان إلى واحد من البروتوكولات التجريبية التالية:

- **المجموعة الأولى (١٠ جرذان):** خضعت الجرذان لنقص الإمداد الدموي لمدة ٣٠ دقيقة.
- **المجموعة الثانية (١٠ جرذان)** :خضعت الجرذان لنقص الإمداد الدموي لمدة ٣٠ دقيقة.وإعادة الارواء لمدة ١٢٠ دقيقة.
- **المجموعة الثالثة (١٠ جرذان):** خضعت الجرذان لنقص الإمداد الدموي لمدة ٣٠ دقيقة.وإعادة الارواء لمدة ١٢٠ دقيقة وتم حقن الفيسفاتين قبل إعادة الإرواء مباشرة.
- **المجموعة الرابعه (١٠ جرذان):** خضعت الجرذان لنقص الإمداد الدموي لمدة ٣٠ دقيقة.وإعادة الارواء لمدة ١٢٠ دقيقة وتم حقن الفيسفاتين بعد ٣٠ دقيقة من إعادة الإرواء.

تم قياس بعض وظائف الجهاز الدوري متمثلة في متوسط ضغط الدم ومعدل ضربات القلب في جميع المجموعات مع بداية نقص الإمداد الدموي وبعد ١٥ دقيقة من نقص الإمداد الدموي و بعد خمس دقائق و ٣٠ دقيقة و ١٢٠ دقيقة من بداية إعادة الإرواء علي التوالي. و بعد فترة إعاده الإرواء المناسبة تم معرفه مدي التلف الناتج في عضله القلب عن طريق قياس الإحتشاء الناتج في البطين الايسر باستخدام صبغه التترازوليم في جميع الفئات التي شملتها الدراسة. وبالإضافة إلى ذلك تم سحب عينات الدم من الجرذان في كل مجموعة لتحليل انزيم اللبنات نازعة الهيدروجين وانزيم كيناز الكيرياتين . وبالإضافة إلى ذلك المالوندای ألديهيد الذي يعتبر واحدا من نتائج عوامل الإجهاد التأكسدي.

وقد أظهرت نتائج هذه الدراسة أن إعطاء الفيسفاتين قبل إعادة الإرواء أو بعد ٣٠ دقيقة من إعادة الإرواء لم يؤثر علي وظائف القلب عند مقارنة جميع المجموعات معاً و أن مستويات انزيم اللبنات نازعة الهيدروجين وانزيم كيناز الكيرياتين و مستوى المالوندای ألديهيد وكذلك حجم الإحتشاء الناتج في البطين الأيسر انخفضت انخفاضاً ذو دلالة إحصائية عندما أعطي الفيسفاتين قبل إعادة الإرواء مباشرة مقارنة بالمجموعة الخاضعة لنقص الإمداد الدموي و إعادة الإرواء.

و من مجمل هذه النتائج يتضح أن التأثيرات الواقية للقلب نتيجه الفيسفاتين تحدث عند التدخل مع بدء إعاده الارواء وليس بعده ويتضح ذلك من تقليل علامات تلف الأنسجة وتقليل حجم الإحتشاء في البطين الأيسر عندما تم حقن الفيسفاتين قبل بداية إعاده الإرواء مباشرة.

لذا، يوصى بمزيد من الدراسات حول الدور الدوائي للفيسفاتين للوقاية أو لعلاج التغيرات الأيضية المصاحبة لأمراض القلب.