Liraglutide attenuate myocardial injury through down-regulation of proinflammatory cytokines: modulates Notch1/Jagged1 signaling pathway


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ABSTRACT

Background: Chronic heart insufficiency and death in acute myocardial infarction (MI), with ST-segment elevation, happens as an effect of abrupt obstruction of the coronary artery and causing the supplied cardiomyocyte to turn into ischemic state, ending in permanent damage and scar tissue formation if circulation is failed.

Methods and materials: A total number of 48 mice were assigned to one of the following 4 experimental groups (n = 8 in each group); Liraglutide pretreated group treated with (3mg/kg intraperitoneal dose of PF) 30 min before ischemia. Assessment of cardiac function by transthoracic echocardiography, the heart tissue and blood were collected and prepared for lab investigation. Results: In this study, the histological features of Liraglutide pretreated mice showed mild architectural alterations and score 2 with mild monocyte infiltration and without hemorrhage (P < 0.05). The %FS was significantly higher at 3 days after surgery in Liraglutide treated mice than I/R control and vehicle treated mice. Liraglutide treated mice had lower levels of MCP-1 in myocardial tissue and plasma P <0.05. Furthermore, plasma myocardial injury marker (cTn-I) level is associated with overexpression of pro-inflammatory mediators after I/R. Conclusions: over expression of inflammatory mediators following I/R suppress LV function; Notch-1 and Jagged-1 protein are down-regulated following I/R; and the administration of liraglutide attenuated chemokine and cytokines through up-regulation of Notch-1 and Jagged-1 activation signaling pathway leading to improved left ventricular function

Keywords: Liraglutide, Chemokine/cytokines, Notch1/Jagged1, LV function
INTRODUCTION

Chronic heart insufficiency and death in acute myocardial infarction (MI), with ST-segment elevation, happens as an effect of abrupt obstruction of the coronary artery and causing the supplied cardiomyocyte to turn into ischemic state, ending in permanent damage and scar tissue formation if circulation is failed [1]. Myocardial I/R injury points to harm of the myocardium which occurs because of the interaction between contents that accumulate during ischemia and those which are conveyed on the subsequent restoration of blood flow. Despite the fact that the organization of reperfusion after MI will produce a noticeable reduction in mortality on account of MI-related heart failure, there is an important increase in patients remaining alive with chronic cardiac dysfunction which resulted from I/R injury [2]. Ischemic heart disease HD represents one of the major reasons of death worldwide. Early blood restoration to the ischemic area considers the most efficient method for the treatment of patients having ischemic heart disease [3]. Ischemic phase for a long time can result in irreversible myocardial injury. Furthermore, the condition does not recuperate but deteriorate after myocardial reperfusion, causing Electrophysiologic, metabolic, and ultra-structural myocardial damage [4]. At the same time as it is crucial to re-institute blood flow at the earliest possible opportunity, reperfusion injury will be sustained. Several signaling routes are accountable for inducing reperfusion injury, and aggravating ischemic damage of the myocardial tissue [5]. So that, novel exploration of pharmacological agents to aid rescue I/R damaged cardiac tissue can allow advantageous clinical outcomes for MI patients. Cytokines synthesize by many cell types, but the major producers are macrophages and helper T cells (Th). Cytokine represents a general name; there are other names include monokine (cytokines produced by monocytes), lymphokines (cytokines produced by lymphocytes), and interleukin (cytokines produced by one leukocyte and acting on other leukocytes) and chemokine (cytokines with chemotactic activities). Cytokines may have autocrine action that means they can act on the cells that secreted by, on nearby cells (paracrine action), or in some situations on distant cells (endocrine action) [6]. A kind of cytokines is famous to induce chemotaxis which is a particular subgroup of structurally related cytokines and known as chemokines [6]. These agents indicate a family of low m.w secreted proteins that possess a variety of functions but mainly function in the migration and activation of leukocytes. Chemokines have preserved cysteine residues that permit them to be classified to four groups: C-C chemokines (monocyte chemoattractant protein or MCP-1, monocyte inflammatory protein or MIP-1α, and MIP-1β), C-X-C chemokines (IL-8 also called growth related oncogene or GRO/KC), C Chemokines (lymphotactin), and CXXXC Chemokines (fractalkine) [7]. In general, the CC chemokines are strong attractants for mononuclear cells, whereas CXC Chemokines are strong Neutrophil chemoattractant [8]. Lately, an increased inflammatory response was recognized as being necessary for the pathophysiology of AMI [9]. Tumor necrosis factor-alpha (TNF-α), the proinflammatory cytokine which synthesized by inflammatory cells like monocytes, neutrophils, and macrophages has the ability to increase inflammatory response and invigorate cytokine secretion [10]. TNF receptor deficiency during I/R worsen ischemic injury and myocyte apoptosis in mice models [11]. TNF-α is able to expand the phagocytosis of neutrophils, encourage the secretion of IL-1 and IL-6 by endothelial cells, and intensify the adhesion of neutrophils and endothelial cells, so promoting local inflammatory development and the response to AMI [12]. Additionally, TNF-α may stimulate macrophages and monocytes to secrete IL-1,
resulting in more induction of the production of TNF-α [13]. In heart, Notch signaling is a key mechanism of normal morphogenesis and is needed for the formation of the atrioventricular canal and valves, for growth and differentiation of the epicardium, endocardium and myocardium and for outflow tract and coronary vessels. Notch signaling is required during growth and three-dimensional organization of the ventricular myocardium to sustain cardiomyocyte precursor differentiation and proliferation in addition to compaction of the initial trabecular myocardium [14]. Eventually, the Notch pathway is very important for coronary Vasculogenesis, that means the formation of primary vascular basic parts from the epicedral mesenchyme, and angiogenesis, that’s to say the budding of new vessels from pre-existing ones how previously presented [15]. Both phenomena are adjusted by vascular endothelial growth factor (VEGF), which downstream pathway includes up-regulation of Notch/Jagged pathway by endothelial cells [16]. Notch signaling seems a homeostatic regulator of the endothelium, as it can mediate either resistance or proliferation of apoptosis throughout active angiogenesis or inhibit contact and cell cycle arrest during blood vessel stabilization. In the latter phase, Notch also prefers Pericyte recruitment from the mesenchyme and stimulates growth, migration and resistance to apoptosis of vascular smooth muscle cells, so that helping the development of functional blood vessels [17]. Liraglutide is an analogue of human glucagon-like peptide 1 (GLP-1)[18]. The peptide sequence of GLP-1 is identical in human, rat, and mouse [19]. GLP-1 increase heart rate [20] GLP-1 effects on appetite and gastric emptying are regulated to be accountable for weight lowering effect [20]. The different properties of GLP-1 make it specifically appropriate for protecting tissue against ischemia/reperfusion injury [21]. In spite of GLP-1R is expressed in autonomic nuclei within CNS that control functions of cardiovascular system [22], the definite cellular localization, associated abundance, and functional significance of the GLP-1R in cardiovascular tissues still have not been fully defined [23]. Other studies have reportedly explained that Liraglutide may markedly decrease the expression Liraglutide was reportedly shown to reduce infarct size and cardiac rupture and improve cardiac output in normal and diabetic mice [24]. Previous studies explained that liraglutide increases changes in markers of inflammation and endothelial function [25]. The current study aimed to study the possible role of liraglutide in attenuated myocardial injury following I/R through Notch 1/Jagged 1 pathway.

Methods

Animals and their preparation

Adult (4 - 6 months) male Albino-Webster mice and their weights ranged from 25 to 38 gram obtained from the College of Science, Babylon University. Mice were acclimated for 14 days in a 12:12-hours light-dark cycle with free access to water and regular chow diet before the experiments in animal house of Kufa University and this investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996). The mice could acclimatize in plastic cages in a controlled temperature (25±1 C) room the temperature within the cage was monitored and maintained near the thermo neutral zone for mice [25] with 60-65% humidity.

Method of left coronary artery ligation
Induction of myocardial ischemia and reperfusion was performed as described previously [11]. In brief, mice (27 to 38 gm body weight) were anesthetized by intraperitoneal injection with a mixture of ketamine (100 mg/kg) and xylazine 5 mg/kg [26]. Animals were intubated with a 20-gauge polyethylene catheter and were ventilated with a rodent ventilator (Harvard Apparatus). A median sternotomy was performed, the left anterior descending artery was identified anatomically, and 8-0 silk suture size was passed around the artery and subsequently tied off for about 30 minutes. Infarction was evident from discoloration of the left ventricle (LV). Finally, the chest wall was closed. The animals remained in a supervised setting until fully conscious.

**Experimental Protocol**

A total number of 48 mice were assigned to one of the following 4 experimental groups (n = 8 in each group):

1. Sham group a total of eight mice underwent a sham surgery, in which mice were anesthetized and involved the identical surgical procedure without the coronary artery ligation.

2. Control I/R underwent 30 minute of LAD ligation followed by 72 hours of reperfusion.

3. I/R + vehicle group received normal saline (the vehicle) and underwent 30 minute of LAD ligation then 72 hours’ reperfusion.

4. I/R + Liraglutide pretreated group treated with (3 mg/kg intraperitoneal dose of PF) 30 min before ischemia and underwent 30 minute of LAD ligation followed by 72 hours reperfusion.

5. All treatments were performed in the morning and followed for survival for three days as mentioned above. After analysis of cardiac function, the heart tissue and blood were collected and prepared for analysis.

**Echocardiography**

Transthoracic echocardiography was performed with a FFsonic 8900 (Fukuda Denshi-Japan) with a 10-MHz phased-array transducer at 72h after I/R. The mouse is injected intraperitoneally with ketamine (100 mg/kg). Heart rates are monitored and generally maintained at 400–500 beats per minute and the chest hair is shaved. ECG needle leads are connected to the limbs for electrocardiogram gating. The mouse is then placed on a warm pad to keep the body temperature around 37°C. Warmed echo gel is placed on the shaved chest. The mouse heart is imaged with a 10 MHz linear transducer LV internal dimensions at end systole and end diastole (LVESD and LVEDD) were measured digitally on the M-mode tracings and averaged from 3 cardiac cycles. LV fractional shortening (%FS) was calculated as [(LVEDD-LVESD)/LVEDD] ×100.

**Collection of samples**
The blood was drawn using direct needle puncture of the heart. For plasma collection, heparin was used as anticoagulant, the samples were stored at 4°C, centrifuged at 4700 × g for 10 min at 4°C. The plasma stored at −20°C until used for further analyses. For cardiac tissue specimens, mice were anaesthetized with ketamine (100 mg/kg), and then killed by injection of 10% KCl to stop the heart at diastole. The heart was excised and weighed and sectioned transversely into two parts from atrial-ventricular junction. The upper parts (atrial) were rapidly frozen and used for morphometric analysis of infiltration of inflammatory cells by ELISA and lower parts (ventricular) for histological examination through staining with hematoxylin and eosin (H&E).

Assessment of inflammatory cell infiltration into the infarct area

Histological sections of the infarcted area were evaluated to assess the degree of accumulated polymorph nuclear Neutrophils and macrophages, because it has been reported that these are important sources for chemokine and cytokine induction or production. The cardiac tissue samples were fixed in 4% paraformaldehyde for 24 h, as described previously [26, 27]. Briefly, cardiac tissue sections (slices) 5μm in thickness were paraffin embedded according to the standard procedure. The degree of heart damage was analyzed by hematoxylin and eosin (H&E) stain, and photographs were obtained from each heart section (n = 3 sections per heart) under optical microscopy. To semi-quantify the difference in cardiac damage, stained histological sections were examined and scored according to the protocol of Zingarelli [28] was used. According to this scoring protocol the following criteria were used: score (0), normal tissue; score (1) mild, interstitial edema and focal necrosis; score (2) moderate, myocardial cell swelling, diffused necrosis; score (3) severe, the presence of ischemia, neutrophil accumulation; and score (4) highly severe, the presence of contraction bands, leukocyte infiltrate, ischemia, and hemorrhage.

ELISA

The freeze parts of myocardial tissue treated in PBS containing 0.5% Triton X100 with a protease inhibitor cocktail, tissue was homogenized and the supernatant used to quantify the chemokine and cytokines (MCP-1, TNF-α, IL-1β, and IL-6) in both plasma and myocardial tissue according to instructions of commercial ELISA kits (Boster, CA), in addition to the plasma cardiac Troponin-I (cTn-I) according to the instruction of commercial ELISA kits (Cloud &Clone, USA). The spectrophotometry of microplate reader (Bio-Rad Laboratories, USA) was used to determine the absorbance of standards and samples at 450 nm. All obtained data were plotted against the linear portion of a standard curve [29].

Statistical analysis

Statistics were performed with the SPSS statistics program (windows version 9.0). To evaluate whether observed differences were significant, paired or non-paired t tests were used when appropriate. A p value (two sided) of less than 0.05 was significant.

Results

Liraglutide increased cardiac function following I/R
We determined whether liraglutide improved cardiac function in mice following I/R by using Echo. The measurements of LVEDD, LVESD, and %FS were similar between I/R control and vehicle treated mice. Furthermore, LVEDDs (mm) was 4.110 (P<0.05) liraglutide treated mice. While, LVESDs (mm) was 3.050.12 (P>0.05) in liraglutide treated mice. Moreover, %FS was significantly higher at 3 days after surgery in liraglutide treated mice than I/R control and vehicle treated mice, as shown in Figure 4C (26.6±2.5% liraglutide, versus 21.2±2.6% I/R control, 21.5±2.7% vehicle treated mice P<0.05).

Table 2: Echocardiography measures were obtained from a short-axis view at the level of the papillary muscle. Control I/R and vehicle mice displayed significantly reduced left ventricle (LV) function, including LVESDs, LVEDDs (mm), ejection fraction and cardiac output, compared with sham mice. Treatment with Liraglutide is improved LV function

<table>
<thead>
<tr>
<th>Echo Measures</th>
<th>Sham</th>
<th>Cardiac ischemia/reperfusion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>538 ± 6</td>
<td>459 ± 3</td>
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<tr>
<td>LVESDs (mm)</td>
<td>4.5 ± 0.02</td>
<td>1.6 ± 0.05*</td>
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<tr>
<td>LVEDDs (mm)</td>
<td>5.1 ± 0.03</td>
<td>1.9 ± 0.11*</td>
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<tr>
<td>Ejection fraction, %</td>
<td>63.5 ± 1.1</td>
<td>24.3 ± 1.8*</td>
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<tr>
<td>FS %</td>
<td>39 ± 1</td>
<td>21.2±2.6%*</td>
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<tr>
<td>Cardiac output, ml/min</td>
<td>5.8 ± 0.3</td>
<td>2.8 ± 0.5*</td>
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The above data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment; ¥P>0.05 versus Liraglutide treated mice. Our previous lab study [30] showed that the pro-inflammatory chemokines (MCP-1) elevated after I/R and plays a substantial role in the accumulation of monocytes to the injured myocardium. In our study, we investigated the effects of Liraglutide in neutralization of MCP-1 and subsequently, reduced the infiltration of accumulation of monocytes and macrophages in myocardium following I/R. In comparison to sham greater level of MCP-1 expression found in both control I/R and vehicle treated mice group moreover; following 72hs of reperfusion the levels of MCP-1 expressing in both plasma and myocardium markedly lower in treated mice with Liraglutide (Figure 1.)
Figure 1: Level of MCP-1 was analyzed by ELISA 72 hrs after reperfusion

Liraglutide treated mice had lower levels of MCP-1 in myocardial tissue and plasma as in figure 1. Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment.

We next investigated the importance effects of Liraglutide on the cardiac tissue and systemic pro-inflammatory responses during I/R. At the end of the experiment (72 hrs. after reperfusion), the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in myocardial tissue and plasma are measured by ELISA according to manufacture protocol. Comparison with I/R and vehicle treated mice, Liraglutide treated mice exhibit greater reduction in the levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in both myocardium and plasma as in figure 2.
Figure 2: Liraglutide reduced the level expression of proinflammatory cytokines in both myocardium figure (A) and plasma figure (B)

Data are expressed as mean ± standard error, n = 8 in each group; *P < 0.05 versus corresponding sham; #P < 0.05 versus control I/R and vehicle treatment. Furthermore, plasma myocardial injury marker (cTn-I) level is associated with overexpression of pro-inflammatory mediators after I/R. The presented data show that the cTn-I is related with elevated of MCP-1 level after I/R corresponding with released of the content of monocyte causing more cardiac injury. Liraglutide have effort reduced cTn-I expression after I/R.

Figure 3: The mean of plasma cTn-I (pg/ml) in the five experimental groups

Data are expressed as mean ± standard error, n = 8 in each group; *P < 0.05 versus corresponding sham; #P < 0.05 versus control I/R and vehicle treatment. Histological, myocardial tissue from I/R and vehicle mice after 72 hrs. of reperfusion period (Figure 5) revealed a marked myocardial injury with the development of contraction bands and polymorph nuclear leukocytes (PMN) infiltration besides interstitial edema and localized extravasation of red blood cells. While the histological features of the Liraglutide treated mice showed mild architectural alterations. To semi-quantify the difference in cardiac damage, histological sections from all groups were examined and scored according to the protocol of Zingarelli [28] was used. According to this score system the following criteria were used: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling; score 3 (severe), the presence of contraction bands and neutrophil infiltrate; and score 4 (highly severe), the presence of contraction bands, leukocyte infiltrate, and hemorrhage. Eight animals in each group were included, and five sections from each animal were evaluated, figure 5.
Figure 5: Ischemia reperfusion in the myocardium

Heart tissues were embedded and cut into sections (5-µm thick). Myocardial ischemia was examined by H&E staining. Scale bar, 100 µm. Magnification: x40 and x100. Demonstrating extensive contraction band change with margination of poly-morph nuclear leukocytes (PMN) in I/R control and vehicle treated mice. While the histological features of the Liraglutide treated mice showed mild architectural alterations.

Figure 6: Zingarelli system indicate that the damage score was significantly reduced in liraglutide treated mice compared with the I/R mice after 72 hrs reperfusion. Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment.

Discussion

The mechanisms accountable for I/R injury have been greatly scrutinized. The main causal factors of cardiac I/R injury are excessive reactive oxygen species (ROS) production, calcium overload, and inflammatory factors release [31]. Finally, all of these factors participate in the death of cardiac cells, by necrosis and apoptosis those results in a functional decline of myocardial tissue [32]. Equally in both, human beings and animal patterns, following 20 min ischemia causes an unalterable cardiomyocyte injury. Better survival rates and salvage of viable myocardium is gained with the earlier blood flow restoration and the best intervention is within the first 2 hours [33]. Throughout ischemia, energy production by mitochondria is reduced and this go along with lowering of intracellular pH consequent to increased production of lactic acid following as a result of anaerobic glycolysis. PH reduction results in upset of ionic homeostasis and intracellular Ca2+ overload. This change happens during ischemia as the cell tries to bring back its intracellular PH leading to
activation of sarcolemal Na+/H+ exchanger. In fact, the Na+ that goes into the cell on the Na+/H+ exchanger is not expelled out in an efficient manner due to a decrease in ATP and an increase in phosphate (Pi) prevent the Na+/K+-ATPase. Following these change, the Na+/Ca2+ exchanger, that usually pumps Ca2+ from the cell, is prevented or even reversed so raising [Ca2+]i. In whatever, at reperfusion phase that a much higher increase in [Ca2+]i occurs which generate myocardial stunning and ventricular arrhythmia [34]. During first 6 hours, the liberation of chemoattractant will draw Neutrophils into the infarct zone and these neutrophils migrate into the myocardial tissue during the next 24 hours and cause vascular plugging and release of degradative enzymes and much more ROS [35]. In spite of the fact that neutrophils stay mainly in the boundary zone, monocytes migrate quickly into the infarct zone [36]. The controlling factor mediating migration of neutrophils into myocardial cells is the reperfusion-dependent induction of interleukin-8 [37]. IL-8 is a chemokine that mediates adhesion, activation, and migration of blood neutrophils (PMN) into inflammatory site [38], when Neutrophils activated by chemoattractant, translocate the β2-integrin Mac-1 (CD11b/CD18) from cellular stores to the plasma membrane and shed L-selectin (CD62L) [39]. Activated leukocytes may harm the vascular endothelium. Thrombomodulin is a recognized marker for endothelial injury, as shown in many clinical findings, and cultured endothelial cells when exposed to activated neutrophils have been shown to release Thrombomodulin [40]. Endothelial injury underlying the observed cardiac Thrombomodulin release caused by oxygen free radicals and proteolytic enzymes liberated by activated leukocytes [41]. In the endothelial cells, eNOS synthesizes Nitric oxide (NO) continuously, where Nitric oxide is a soluble gas that regulates endothelial function and basal vascular tone, and preserves blood oxygenation through hypoxic pulmonary vasoconstriction. Many previous studies have examined the endogenous production of NO, and its therapeutic application in I/R. NO or NO donor’s administration before ischemia diminishes the outcomes of MIRI and so that decreases endothelial dysfunction and infarct size [42]. These beneficial effects of NO are correlated to a pharmacological type of preconditioning [43]. Excessive NO is commonly go along with pain, atherosclerosis, cancer, neurological disorders, inflammation and immune disorders [44]. Increased expression levels of iNOS after myocardial infarction in mice, have been seen that results in the stimulation of excessive NO, declined cardiac function and increased mortality [45]. However, the process of restoring blood flow to the ischemic myocardium can induce myocardial reperfusion injury, which can paradoxically reduce the beneficial effects of myocardial reperfusion. Thus, reperfusion itself may lead to accelerated and additional myocardial injury beyond that generated by ischemia alone [11]. The prompt return of blood to the heart is life-saving; however, reperfusion injury extends myocardial damage beyond that inflicted by the ischemia itself [46]. Although there are clearly multiple mechanisms of tissue damage from ischemia and reperfusion, the blood plays a major role in the inflammatory component of reperfusion injury. Proinflammatory mediators, stimulated by ischemia and reperfusion, activate PMNs and the coronary endothelium. Adhesion molecules are then expressed on the surfaces of both cell types, resulting in multiple cell-cell interactions. PMNs adhere to vascular endothelium and generate toxic free radicals, which induce microvascular dysfunction and blood flow defects, contribute to apoptosis and ultimately extend the myocardial infarction [23]. In the present study, we examined whether liraglutide may reduce myocardial injury following I/R in mice model and possible mechanistic signaling pathway to their pharmacological work. In comparison to sham group, there was significant increase in cardiac tissue and plasma
levels of IL-1β, IL-6 and TNF-α in both controls I/R and vehicle I/R group in the present study. In this study, there was greater level of MCP-1 expression found in both control I/R and vehicle I/R treated mice groups in comparison to sham group as it was analyzed by ELISA for both plasma and homogenized cardiac tissue. Many experimental and clinical studies have demonstrated up-regulation of MCP-1 after MI, with recruitment of monocytes/macrophages to the ischemic myocardium [47], reported that an anti-MCP-1 gene therapy improved survival rate of mice as well as attenuated LV cavity dilatation and contractile dysfunction, interstitial fibrosis, recruitment of macrophages, myocardial gene expression of TNF-α, and transforming growth factor-β, while [48] showed a late increase in plasma MCP-1 levels in subacute phase in AMI patients. In this present study, ELISA analyses for plasma showed that cardiac troponin I (cTn-I) increased obviously in control I/R and vehicle I/R treated groups in comparison to sham group. Several previous studies have shown that cardiac troponins have powerful predictive value for adverse cardiovascular events and death [49] clarified that myocardial damage causes disruption to the membrane integrity of the normal cardiac myocyte and loss of intracellular content into the extracellular space, so that, elevated levels of cytosolic and structural proteins, such as cardiac troponins and CK-MB, can be detected in the blood. By using Echo, the measurements of LVEDD, LVESD, heart rate, ejection fraction, cardiac output and %FS were nearly similar between control I/R and vehicle I/R treated mice and lower than that’s appeared in sham group. The development of HF, following AMI, is associated with “ventricular remodeling”, i.e., that’s changes in cardiac structure and function. Heart rate increased from 450–500 bpm to ~800 bpm in the same mice before myocardial infarction [50] as well as after 8 wk of reperfusion, but in our present study we measure the heart rate after 72 hours of reperfusion show stabilized heart rate of sham group.In this study, the histological examination for myocardial tissue from control I/R and vehicle I/R treated groups after 72 hours of reperfusion period revealed a marked myocardial injury with the development of contraction bands and polymorph nuclear leukocytes (PMN) infiltration besides interstitial edema and localized extravasation of red blood cells indicated that the damage score was significantly increased in comparison to sham group. Other study found that the pathological changes more marked in the myocardial tissues from the rats in control group when compared with the sham group in the rats model study, and these changes involved inflammatory cell infiltration, necrosis and atrophy of the myocardial fibers [50]. These findings support the present results regarding histopathological changes in the reperfused cardiac tissue and are in relevance with [51] who clarified that myocardial I/R injury showed a significant disruption of the myocardial tissue structure described by the presence of extensive necrosis demonstrated the presence of critical myocardial membrane injury, edema and inflammatory cells infiltrates to the ischemic reperfused cardiac tissue compared to sham group by using microscopic examination regarding histopathology [11]. The present study that showed the Notch-1/Jagged-1 had low expression in the injured myocardial cells after I/R corresponding with high expression of inflammatory mediators (P > 0.05) in both control I/R and vehicle I/R treated mice groups. Notch signaling can assist myocardial regeneration, saves the myocardium from ischemia, stimulates angiogenesis, and prevents cardiac fibroblast to myofibroblast transformation (CMT). Each of these events encourages cardiac repairing after myocardial injury [52], while others demonstrated that cardiac-specific Notch-1 knockdown resulted in significant aggravation of I/R injury, as proved by enlarged infarct size, decreased cardiac function, increased apoptotic evidence in the
myocardium and cardiac fibrosis [53]. It clarified that the up-regulation of Notch-1 in the hypertrophic myocardium regulates the adaptive response of the heart to stress conditions, not only by restricting the extent of the hypertrophic response but also by contributing to cell survival in cardiomyocytes [14], and demonstrated that nearly all of the Notch receptors and ligands are found at varying levels in the injured myocyte during post-infarction remodeling, that indicates a regulatory role for Notch signaling in the functional recovery of the ischemic myocardium [54]. In comparison with control I/R and vehicle treated mice, liraglutide treated mice exhibit greater reduction in the levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in both myocardium and plasma in the present study. It is reported that the inflammatory response in macro-phages induced by LPS has been reduced by liraglutide [34]. Also, showed that liraglutide could inhibit systemic inflammation in rats [55], clarified that liraglutide arrests the production of inflammatory mediators in arthritic rats.

Conclusions

Our data suggested that pretreatment with liraglutide decreased infiltration of PMN cells through down-regulation of Notch-1 and Jagged-1 protein and attenuated over expression of proinflammatory mediators. Further study needs to examine the effects of high dose liraglutide shortly after I/R.

Source of Funding: None

Conflict of Interest: None

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