Canadian Journal of Physiology and Pharmacology

Sitagliptin protects Diabetic Rats with Acute Myocardial Infarction Involves Induction of Angiogenesis: Role of IGF-1 and VEGF

Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2018-0670.R2
Manuscript Type:	Article
Date Submitted by the Author:	06-Apr-2019
Complete List of Authors:	Khodeer, Dina; Faculty of Pharmacy, Suez Canal University, Pharmacology and Toxicology; Bilasy, Shymaa ; Suez Canal University Faculty of Pharmacy Farag, Noha; Faculty of Medicine, Suez Canal University, Physiology Mehana, Amir; Suez Canal University Faculty of Science Elbaz, Amani; Suez Canal University Faculty of Medicine
Is the invited manuscript for consideration in a Special Issue:	Not applicable (regular submission)
Keyword:	Diabetes Mellitus, Myocardial Infarction, Sitagliptin, Rats, VEGF

SCHOLARONE™
Manuscripts

Sitagliptin protects Diabetic Rats with Acute Myocardial Infarction Involves Induction of Angiogenesis: Role of IGF-1 and VEGF

Dina M. Khodeer^{1*}, Shymaa E. Bilasy², Noha E. Farag³, Amir E. Mehana⁴, Amani A. Elbaz³

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

²Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

³Department of Physiology, Faculty of Medicine, Suez Canal University, Ismailia 41522, Egypt

⁴Department of Zoology, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt

³Department of Physiology, Faculty of Medicine, Suez Canal University, Ismailia 41522, Egypt

***Corresponding author:**

Dina M. Khodeer

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

E-mail: dina_khoudaer@pharm.suez.edu.eg

https://orcid.org/0000-0002-3412-4138

Tel : 002-01224055882

Fax : 002-064-3230741

Abstract

Angiogenesis is regulated in a tissue-specific manner in all patients, especially those with diabetes. In this study, we describe a novel molecular pathway of angiogenesis regulation in diabetic rats with myocardial infarction (MI) and the cardioprotective effects of different doses of sitagliptin. Male rats were divided into five groups, normal vehicle group, diabetic group, diabetic+MI, diabetic+MI+5 mg/kg sitagliptin, diabetic+MI+10 mg/kg sitagliptin. Isoproterenol in diabetic rats significantly ($p<0.05$) disturbed the electrocardiography (ECG) pattern, cardiac histopathological manifestations and an increase in inflammatory markers compared to vehicle and diabetic groups. Treatment with sitagliptin improved ECG reading, histopathological sections, upregulated vascular endothelial growth factor (VEGF) and transmembrane phosphoglycoprotein protein (CD34) in cardiac tissues and increased serum Insulin-like growth factor 1 (IGF-1) as well as decreased cardiac tissue homogenate for Interleukin 6 (IL 6) and COX 2. Generally, a relation was found between serum IGF-1 with cardiac VEGF and CD34 accompanied by the improvement of cardiac function in diabetic rats with MI. Therefore, the current effects of sitagliptin were, at least in part, may occur partly through improving angiogenesis and mitigation of inflammation. Consequently, these data suggest that sitagliptin, in a dose-dependent manner, may contribute in defense against acute MI in diabetic individuals.

Keywords: Diabetes Mellitus; Myocardial Infarction; Sitagliptin; Rats; VEGF

Introduction

Diabetes mellitus is a major public health problem that affects 347 million people worldwide (Danaei et al., 2011; Kassahun, Gashe, Mulisa, & Rike, 2016). On the long-term, uncontrolled hyperglycemia is associated with an increased risk of cardiovascular diseases, including myocardial infarction (MI) (Einarson, Acs, Ludwig, & Panton, 2018; Wilson et al., 1998). In MI, irreversible damage in the Cardiac muscle occurs secondary to obstruction/stenosis of the coronary arteries (Davis, Fortun, Mulder, Davis, & Bruce, 2004). Isoproterenol (ISO) is a β -adrenergic agonist that causes increased oxidative stress in the myocardium and necrotic lesions in the cardiac muscle (Chagoya de Sánchez et al., 1997; Piepoli et al., 2016; Zhang et al., 2005). ISO-induced MI in rats imitates alterations in blood pressure level, beats per minute, electrocardiogram (ECG) together with left ventricular malfunction (Yousefi et al., 2013).

The peptide hormone-insulin-like growth factor-1 (IGF-1) has structural and functional homology to insulin. It stimulates bone growth, cell differentiation, and metabolism (Froesch, Zenobi, & Hussain, 1994). Diabetes is usually associated with low serum levels of IGF-1, and several reports have outlined the function associated with IGF-1 changes during the progress of MI and cardiovascular diseases (Donath et al., 1998; Vaessen et al., 2001). Moreover, in an ISO-induced MI in rats, treatment with IGF-1 reduced myocytes necrosis and increased capillary sprouting in the myocardium. The cardioprotective action of IGF-1 during ischemia could be attributed to stimulation of angiogenesis because the levels of the angiogenic cytokine (interleukin-8) were increased (Haleagrahara, Chakravarthi, & Mathews, 2011).

Additionally, it was documented that, vascular endothelial growth factor (VEGF) is a strongly distinct mitogen for vascular endothelial cells (Duffy, Bouchier-Hayes, & Harmey, 2013). The expression of VEGF is potentiated as a result of a number of cytokines, hypoxia or by activated oncogenes (Ferrara, 2004). VEGF triggers endothelial cell proliferation stimulates cell migration and suppresses apoptosis. Moreover, VEGF induces angiogenesis along with permeabilization of blood vessels and represents the main role on the management of vasculogenesis (Neufeld, Cohen, Gengrinovitch, & Poltorak, 1999). On the other hand, transmembrane phosphoglycoprotein protein (CD34) is a member of single-pass transmembrane sialomucin family proteins, which often used as a direct marker for measuring the degree of neoangiogenesis by identifying the microvasculature vessel density (Inda et al., 2007).

Incretins are gastrointestinal hormones, which enhance endogenous insulin secretion and reduce glucagon secretion, resulting in decreased blood glucose after food consumption (Cernea & Raz, 2011; Gautier, Fetita, Sobngwi, & Salaün-Martin, 2005). The incretin effect is attributed mostly to two hormones; a glucose-dependent insulintropic peptide secreted from the L-cells of the distal ileum and colon, and glucagon-like peptide-1 (GLP-1), emitted from the K-cells in the duodenum and jejunum (Mortensen, Christensen, Holst, & Orskov, 2003). In type 2 diabetes, GLP-1 concentrations are lower than in healthy individuals, implying its role in the pathogenesis of the disease (Zander, Madsbad, Madsen, & Holst, 2002). It exerts its insulintropic effect via binding to GLP-1 receptors (GLP-1R), which are expressed in a variety of extra-pancreatic tissues including the heart and vasculature in both humans and rodents (Bullock, Heller, & Habener, 1996; Nyström et al., 2004). Interestingly, mice with inherited deletion of GLP-1R (GLP-1R^{-/-}) display increased the thickness of the left ventricle and impaired left ventricle contractility and diastolic function (Gros et al., 2003). On the other hand, Baggio and his co-author (2018) validated that the GLP-1R transcripts were also detected by in situ hybridization in human cardiac sinoatrial node tissue. Moreover, transient GLP-1 administration improved the cardiovascular outcomes in patients with MI (Nikolaidis et al., 2004). Nevertheless, GLP-1 is rapidly degraded by the dipeptidyl peptidase-4 (DPP-4) enzyme, resulting in a short circulating half-life of the hormone and thus limiting its therapeutic applicability (Field, Chaudhri, & Bloom, 2009).

Sitagliptin is an oral anti-hyperglycemic agent that prolongs the availability of the endogenous GLP-1 levels by inhibiting its rapid metabolism by the DPP-4 enzyme (Herman, Stein, Thornberry, & Wagner, 2007; Kim et al., 2005). The cardioprotective effects of DPP-4 inhibitors are controversial, and their mechanisms are unclear. Previous studies indicated that sitagliptin mediated DPP-4 inhibition improved the cardiovascular outcomes in experimentally induced MI in mice (Sauvé et al., 2010) and in humans with coronary artery disease (Read, Khan, Heck, Hoole, & Dutka, 2010). Additionally, in a mouse model of hindlimb ischemia, sitagliptin treatment enhanced the blood flow to the blocked arteries of the lower extremities (ischemic limb) through increasing the levels of endothelial progenitor cells, CD34 expression and thus improving neovasclogenesis (C.-Y. Huang et al., 2012). Recent publication indicated that DPP-4 inhibition by saxagliptin failed to improve the cardiovascular outcomes among diabetic patients, although it successfully controlled hyperglycemia (Scirica et al., 2013).

Thus, in this study, we aimed to investigate the hypothesis if sitagliptin protects diabetic rats with acute myocardial infarction involving induction of angiogenesis with clarifying the role of IGF-1 and VEGF

Methods

Animals

Forty male albino rats with a mean weight 150 g were housed in spacious wire mesh cages with free access to standard rat chow diet and tap water. Rats were housed in a normal light-dark cycle at around 22 °C. The experimental protocols were accepted by the Animal Care Committee at Faculty of Pharmacy, Suez Canal University.

2. Drugs and chemicals:

Both Streptozotocin (STZ) and Isoproterenol hydrochloride were purchased from Sigma-Aldrich (Germany), STZ was prepared in a citrate buffer (0.1 M, pH = 4.5). Sitagliptin (Januvia® tablet) was purchased from Merck Sharp & Dohme Corp. (a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA) and was prepared as a suspension in 1% Na-CMC.

3. Induction of type 2 diabetes and acute myocardial infarction:

A high-fat diet was served to the rats for four weeks. Fasting and random blood-glucose levels were recorded every three days. After four weeks, a freshly prepared streptozotocin (30 mg/kg, i.p.) in a volume of 1 ml/kg animals were injected in overnight fasted rats (Skovsø, 2014). Three days after STZ administration, the blood-glucose levels in each rat were determined using the One-Touch Ultra Mini glucometer (USA). Rats which had fasting blood-glucose levels higher than 135 mg/dl were regarded as diabetic and involved in the experiment. Then, acute MI (AMI) was induced by injection of two subsequent doses of isoproterenol per two days (85 mg/kg/day, subcutaneous) (Li et al., 2010).

4. Experimental design:

In this study, a total of 40 rats were randomly allocated into five groups, eight rats each. Group I: Rats fed with a normal palatable diet, received citrate buffer (1 ml/kg, i.p.) at the end of week 4 [control to STZ] and two sequential dosages of saline (vehicle of isoproterenol) at fifth week.

Group II: Rats fed with a high fat diet (HFD) for four weeks followed by STZ in dose (30 mg/kg), then, rats were injected with two sequential dosages of saline (vehicle of isoproterenol) at fifth week.

Group III (Diabetic+MI group): High fat diet and STZ-induced diabetic rats, injected with two sequential subcutaneous dosages of isoproterenol (85 mg/kg/day) at fifth week.

Group IV: Diabetic+MI rats treated with sitagliptin (5 mg/kg/day, P.O).

Group V: Diabetic+MI rats treated with sitagliptin (10 mg/kg/day, P.O).

Generally, starting from the first day of isoproterenol injection, sitagliptin (5, 10 mg/kg/day, P.O) doses were given daily by gastric gavage for four weeks (Yang et al., 2016). The body weight of the rats was monitored every week.

5. Electrophysiology

Forty-eight hours after the second dose of isoproterenol hydrochloride, all animals were anesthetized, and ECG was recorded using research Biopac Data Acquisition Device; MP150 (BIOPAC Systems, Inc., USA). Needle electrodes were inserted subcutaneously for limb leads (Lead II). The assessed ECG parameters were; heart rate (beats per minute), QRS complex (msec), QT interval (msec), ST segment (msec), QTc interval and R amplitude (volt).

6. Blood sampling

After ECG recording, a midline incision was made, and a 2 ml blood sample was withdrawn from the heart for biochemical analysis. The blood samples were centrifuged for 15 min. at $2000 \times g$ immediately after collection. The serum was separated and stored at -80°C till further usage.

7. Processing of the heart

At the termination of the study protocol, the great vessels and atria were detached. Afterward, the heart was taken out and washed with ice-cold phosphate buffered saline. The hearts and left ventricles (LV) were weighed, and the ratio to body weight was calculated to assess cardiac hypertrophy. Subsequently, each ventricular myocardium was split up into two parts: The first part was stored for further biochemical analyses, and the second part was fixed overnight at 4 % paraformaldehyde and then embedded with paraffin. All paraffin-embedded tissues were sectioned at 4 μm thicknesses at the cardiac apex and left to dry overnight at 37°C . Sections were then deparaffinized, rehydrated and prepared for routine histological staining with hematoxylin and eosin (H&E) and immunohistochemistry for VEGF and CD34.

8. Determination of serum cardiac biomarkers

Serum lactate dehydrogenase (LDH) activity was determined using previously prepared serum samples in accordance with Buhl and Jackson (1978). While serum creatine kinase MB isoenzyme (CK-MB) activity was assayed following the immunoinhibition method (Würzburg et al., 1976).

9. Determination of cardiac levels of IGF-1, IL-6 and COX 2 using ELISA kits.

Cardiac samples were homogenized and added to the enzyme-linked immunosorbent assay (ELISA) kits for insulin growth factor- α (IGF-1), Interleukin-6 (IL 6) and cyclooxygenase 2 (COX 2) (Ray Biotech Inc., Norcross, USA). The assays were conducted following the guidelines of the company using an automated ELISA reader (Europe S.A., Belgium). The samples were measured in duplicate and represented by the mean.

10. Histopathological examination of the heart tissues

The stained tissues were examined by two pathologists; the histopathological changes were graded as 1, 2, 3, and 4 for low, moderate, high, and intensive pathological changes, respectively. The system of scoring was carried out as mentioned previously (Acikel et al. 2005).

11. Immunohistochemistry

Immunostaining was done utilizing a streptavidin-biotin-immunoperoxidase complex technique using four μm thick sections; this was deparaffinized along with heated in citrate buffer solution (0.01 M, pH 6.0) for 15 min by using a microwave oven to access antigens. Mouse CD34 monoclonal antibodies and rabbit VEGF polyclonal antibodies were obtained from Thermo Fischer Scientific (Germany). Sections were incubated at 4 °C for 24 hours with the corresponding primary antibody. After that, DAB was applied as the final chromogen, and the nuclei were faintly counterstained with Mayer's hematoxylin solution to facilitate microscopic assessment. Immunoreactivity for VEGF or CD34 was evaluated regarding the staining intensity, and coverslipping was performed as the final step before slides were examined under a light microscope (x 40) (Olympus CX21, Japan).

12. Image analysis

The slides were examined to measure the immunoreactivity to VEGF or CD34 using a computer assisted image analysis system "ImageJ 1.45F" (National Institute of Health, USA). The optical density was calculated on consecutive sections indicative of whole tissue section. The

histological analysis was performed by an experienced pathologist who was blinded to the experimental groups.

2.12. Statistical Analysis

Results were stated as means $\pm$ standard error of the mean (SEM). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. The non-parametric data was tested using the Kruskal-Wallis ANOVA. Data were analyzed using The Statistical Package for the Social Sciences, version 20 (SPSS Software, SPSS Inc., Chicago, USA). Differences between means were considered to be statistically significant at $P < 0.05$.

Results

The effect of sitagliptin on body weight, left ventricles weight (LV) and heart weight (HtWt) to body weight (BWt) ratios:

By the end of the experiment, vehicle group rats exhibited 8.84% increase in BWt. While the diabetic control and diabetic+MI rats exhibited 36.24 % and 35.8% increases in BWt, respectively. Moreover, the diabetic and diabetic+MI groups exhibited significant increases ($P < 0.05$) in LV weight and HtWt to BWt ratio, in comparison of the vehicle group. Treatment with sitagliptin significantly mitigates the increases in BWt, LV weight, and HtWt to BWt ratio as compared to the diabetic and diabetic+MI groups ($P < 0.05$, Table 1).

The effect of sitagliptin on fasting blood glucose

Statistical analysis revealed that diabetic and diabetic+MI rats showed significantly higher fasting blood glucose levels in comparison of the vehicle group ($P < 0.05$, fig 1). Administration of sitagliptin (5 or 10 mg/kg) significantly decreased fasting blood glucose as compared to diabetic or diabetic+MI control groups (fig 1). Further, treatment with a higher dose of sitagliptin (10 mg/kg) resulted in a significant ($P < 0.05$, fig 1) decrease in fasting blood glucose level in comparison with a lower dose sitagliptin (5 mg/kg) treated group.

The effect of sitagliptin on ECG pattern

By the end of the experiment, the control group displayed a normal ECG pattern with normal HR, QRS complex, QT, QTc intervals and R amplitude. Whereas the diabetic and diabetic+MI groups showed a significant increase in HR, ST segment, QT interval, QTc interval, and R amplitude, as well as a significant decrease in QRS complex compared with the vehicle

group. These changes were more significant in the diabetic+MI group than in the diabetic group ($P<0.05$, Fig. 2a and b). The observed changes in ECG parameters (HR, ST segment, QRS) in the diabetic and diabetic+MI groups were significantly ameliorated after treatment with sitagliptin (5 mg/kg). Further, low dose of sitagliptin (5 mg/kg) significantly ($P<0.05$, Fig. 2a and b) decreased QT and QTc intervals in comparison with diabetic+MI group. While, higher dose of sitagliptin (10 mg/kg) normalized all the ECG terms when compared to the diabetic and diabetic+MI groups ($P<0.05$, Fig. 2a and b)

The effect of sitagliptin on cardiac enzymes

Treatment of diabetic rats with isoproterenol significantly increased cardiac serum LDH and CK-MB levels as compared to both untreated diabetic and vehicle control groups. Treatment with sitagliptin (5 mg/kg) was not associated with significant reductions of serum cardiac enzymes compared with diabetic+MI group, while the higher dose of sitagliptin (10 mg/kg) significantly ($P<0.05$, Table 2) decreased the levels of serum cardiac enzymes (LDH and Ck-MB) in comparison with the diabetic+MI group.

Effects of sitagliptin on the level of IGF-1, IL 6 and COX 2

The diabetic group rats exhibited significantly lower IGF levels as compared to the vehicle group (fig 3). While treatment with isoproterenol in diabetic rats resulted in a significant increase in IGF when compared with the diabetic group. Nevertheless, sitagliptin treated groups revealed a significant increase in IGF compared to both diabetic and diabetic+MI treated group ($P<0.05$, fig 3). Further, the diabetic group exhibited a significant increase in cardiac tissue IL-6 and COX-2 levels, compared with the vehicle group. Moreover, the diabetic+MI group produced a significant increase in IL 6, and COX 2 compared to both vehicle and diabetic groups (fig 4, 5). Sitagliptin (5 or 10 mg/kg) treated groups exhibited significant decreases in IL-6 and COX-2, compared to diabetic+MI group ($P<0.05$, fig 4&5).

Histopathological examinations of cardiac tissues subjected to acute MI

Assessment of LV myocardial tissue in the vehicle group showed that the myocardial fibers were typically arranged with obvious striations and no recognizable degeneration. While histological sections in the LV of diabetic or diabetic+MI groups revealed average to severe necrotic changes, in particular, the cardiomyocyte sarcoplasm was densely eosinophilic, nuclei

exhibited a reduction in size and condensation, and there was an increase in some neutrophils and lack of striations (Fig. 6). Considerably, treatment with sitagliptin lowered the mean fibrosis grade in comparison to a diabetic or diabetic+MI group ($P<0.05$, fig 6&7).

Effect of sitagliptin on immunohistochemical staining (VEGF, CD34) in cardiac tissues

Figures 7 and 8 demonstrate images for cardiac tissue sections stained for VEGF and CD34. The immunohistochemical staining exhibited higher cardiac tissue levels of VEGF or CD34 in isoproterenol-treated rats as compared to the diabetic control group ($P<0.05$, Fig. 8a&b, 9a&b). The groups treated with sitagliptin (5 or 10 mg/kg) showed a significant increase in the expression of VEGF or CD34 compared with the diabetic group ($P<0.05$, Fig. 8a&b, 9a&b). The effect of the higher dose of sitagliptin (10 mg/kg) was more potent than that of those of lower dose (5 mg/kg).

Discussion

This research was performed to investigate the protective effects of sitagliptin in diabetic rats with AMI and whether these effects involve the induction of angiogenesis via IGF-1 and VEGF. Therefore, a diabetic rat model was established through HFD supplementation and a small dose of STZ. Diabetic rats exhibited several alterations in the histopathological picture, ECG pattern, and serum levels of cardiac enzymes. These results are in agreement with a former study that suggested DM as a chief risk factor for the development of cardiac pathology (Deniz et al., 2015; Di Paola et al., 2018). Furthermore, it was suggested that the electrocardiographic changes in DM patients are not specific and that they are particularly caused by an increased tone of the sympathetic nervous system what was indirectly confirmed by the heart rate variability findings in these patients (Kittnar, 2015). Similarly, several mechanisms have been implicated in the pathogenesis of diabetic cardiomyopathy which include Changes in myocardial structure, left ventricular hypertrophy, alterations in calcium signaling and metabolism as uncoupling of oxidative phosphorylation, all of that were described mainly in animal models (Boudina & Abel, 2010).

Moreover, STZ has been shown to have direct effects on muscle fibres that promote diabetic myopathy, as within STZ-treated myoblasts, it increases reactive oxygen species that was associated with significant G₂/M phase cell-cycle arrest (Johnston, Campbell, Found, Riddell, & Hawke, 2007).

On the other hand, the ECG alterations in isoproterenol-treated rats were mainly attributed to generation of free radicals which are unifying components in most forms of cardiovascular diseases (CVDs) especially MI (Goyal et al., 2010; Hassan et al., 2016), as well as, associated with the decreased contractile capacity of cardiac ventricles (Erickson et al. 2013). The alteration in ST-interval is a very sensitive marker for MI and refers to myocardial necrosis (Khodeer, Zaitone, Farag, & Moustafa, 2016). Nevertheless, the QT interval represents the time pertaining to the repolarization of the ventricles. Therefore, the QT interval prolongation, observed after isoproterenol treatment indicates energy depletion in the ischemic myocardium. Additionally, QRS interval reflects declined conduction through ventricles (Khodeer et al., 2016).

Diabetes mellitus was reported to promote the progression of MI by inhibiting the Notch signaling pathway that regulates cell proliferation, death, differentiation, cell-to-cell communication and angiogenesis. Activating the Notch pathway has been reported to alleviate I/R and hypoxic injuries (Wu, Yu, Zhang, & Zhang, 2017). Although angiogenesis plays a role in the pathogenesis of diabetic microvascular complications in some tissues like the retina, DM impairs the formation of small blood vessels in other tissues like the heart and skin (Cheng & Ma, 2015).

Recently, myocardial angiogenesis has been recognized as an innovative therapeutic approach for MI (Mitsos et al., 2012). The paradigm to improve therapeutic angiogenesis has focused on enhancing the formation of neovessels from preexisting, terminally differentiated endothelial cells to accelerate neovascularization (F. Huang et al., 2017). Additionally, It has been shown that endothelial progenitor cells (EPCs), precursors derived from bone marrow, can differentiate into endothelial cells and incorporate into areas of neovascularization (Balaji et al., 2015). Further, EPCs can move to ischemic sites and contribute to neovascularization under the effects of VEGF, resulting in reduction of the infarct size (F. Huang et al., 2017).

The current study showed that in a dose-dependent manner, treatment with sitagliptin significantly improved the ECG findings, histopathological abnormalities, cardiac enzymes and body mass index changes. The present results confirm previous results that sitagliptin alleviated myocardial remodeling of the LV and improved cardiac dysfunction in diabetic rats (Liu et al., 2015), as well as it could protect against ventricular arrhythmia (T.-M. Lee, Chen, & Chang, 2016).

Another study reported that sitagliptin treatment augmented the number of circulating angiogenic cells in a rat model of critical limb ischemia (Chua et al., 2013). Moreover, Dai et al. indicated that sitagliptin-induced preservation of circulating endothelial progenitor cells (EPC) angiogenic function can improve blood perfusion and angiogenesis. This is probably mediated by sitagliptin-induced protection against EPC apoptosis by boosting autophagy (Dai et al., 2017). Furthermore, EPCs, known as an alternative cell source for participating in angiogenesis process, have also putative potency to mobilize, circulate and recruit to sites of neovascularization in response to chemotactic factors and cell surface markers including CD133, CD34 and VEGFR-2, Tie-2, etc (Povsic et al., 2009; Rae, Kelly, Egginton, & St John, 2011). Additionally, Tie-2 receptors which control the mobilizations of EPCs, are controlled by two ligands angiopoietin I (Ang I) and angiopoietin II (Ang II) (Siavashi et al., 2016), Ang I induced and stabilized tube-like structures via EPC intracellular Tie2-FAK-AKT complex and subsequent changes in pp38, pSAPK/JNK, and phosphorylated extracellular signal-regulated kinases mediated Mitogen-activated protein kinases activation (Moon et al., 2015). However, The density of the cells came by activation of CD34 is increased by upregulation of Ang II (Siavashi et al., 2016) as well as it was showed to activate mobilization of EPCs in ischemic condition by exocytosis (Kuo et al., 2008). In addition, it was found a positive correlation of VEGF, Ang II and SDF-1 with EPC levels in septic patients indicates an impact of those factors on mobilization of EPC from the bone marrow during sepsis (Patry et al., 2018).

Likewise, sitagliptin was shown to increase the mean insulin content of islet grafts and area of insulin-positive tissue, as well as β -cell proliferation (Aston-Mourney et al., 2013). Moreover, it markedly increased endothelial cell proliferation, microvessel density, and blood flow, as well as upregulation of VEGF expression and circulating angiogenic cell numbers, angiogenesis and blood flow in the rat critical limb ischemia area (Chua et al., 2013). In vivo, data demonstrated that sitagliptin treatment phosphorylated cAMP response element-binding (CREB) and induced islet vascularization through VEGF-A/VEGFR-2 signaling pathway (Samikannu et al., 2013). Furthermore, sitagliptin showed to inhibit the biological inactivation of SDF-1 (stromal cell-derived factor-1), increase EPCs mobilization and incorporation, which, in turn, may regenerate capillaries and reduce myocardial ischemia induced by strenuous exercise (Fiordaliso et al., 2016). All of these previous studies agree with the present results which demonstrated that sitagliptin induced a significant increase in VEGF, CD34 expression and it is

clear that, sitagliptin may induce angiogenesis via EPCs correlated with Ang II, VGAF and CD34 expression pathway, as a novel molecular pathway for cardiovascular protection in diabetic animals.

The current study showed that treatment with sitagliptin induced a significant increase in IGF-1 in a dose-dependent manner. The IGFs play a significant role in the physiology of endothelial cells by tube formation, promoting migration and nitric oxide production (Bach, 2014). These events are intermediated by the IGF1 and IGF2/mannose 6-phosphate receptors and are controlled by a family of high-affinity IGF binding proteins (Kolluru, Bir, & Kevil, 2012). Furthermore, IGFs also increase the number and function of EPCs, which may contribute to protection from atherosclerosis, as well as promoting angiogenesis (Bach, 2014). Moreover, it was proven that in endothelial cells, IGF-1 stimulates the expression of angiogenesis-related growth factors with the activation of the PI3-kinase/Akt signaling pathway (S. Lin et al., 2017).

Additionally, the present study showed that sitagliptin induced a significant decrease in inflammatory markers IL-6 and Cox-2 tissue levels. These results come in line with previous findings that treatment with sitagliptin downregulated the mRNA levels of IL-6, COX-2 and iNOS in lipopolysaccharide-stimulated cardiomyocytes in a dose-dependent manner (S.-B. Lee, Lee, Shin, Jang, & Lee, 2017; S.-H. Lee et al., 2016). Furthermore, sitagliptin inhibited the increased protein expression of IL-6, TNF- α and IL-1 β . Similarly, NF- κ B mRNA expression was reduced, and its translocation to the nucleus was suppressed by treatment with sitagliptin (C.-H. Lin & Lin, 2016).

Thus, the present study highlighted the cardioprotective role of sitagliptin in diabetic rats with MI in a dose dependent manner, focusing on its anti-inflammatory properties, its role in increasing induction of IGF-1 and VEGF expression in myocytes and therefore, enhancing angiogenesis and its glucose lowering action, which by the role improve the cardiovascular tissue function. More studies are required to test sitagliptin against MI in normal animals. Similarly, the action of sitagliptin on other angiogenic markers is remained elusive, and more studies need to be investigated. Generally, sitagliptin is considered to be one of the drugs of the best choice to be used in diabetic patients who suffered concurrently with MI.

The limitation of this study is the use of ECG to confirm the injurious action of isoproterenol, in the initiation of myocardial infarction, and it is recommended to use several other tests like TCC staining to evaluate and confirm that the isoproterenol induce MI. Further knowledge is required to detect the molecular mechanisms of GLP-1R expression, to evaluate all possible mechanisms involved in its expression among ventricular or atrial tissue of heart in rat.

References

- Aston-Mourney, K., Subramanian, S. L., Zraika, S., Samarasekera, T., Meier, D. T., Goldstein, L. C., & Hull, R. L. (2013). One year of sitagliptin treatment protects against islet amyloid-associated β -cell loss and does not induce pancreatitis or pancreatic neoplasia in mice. *American Journal of Physiology - Endocrinology and Metabolism*, 305(4), E475–E484. <https://doi.org/10.1152/ajpendo.00025.2013>
- Bach, L. A. (2014). Endothelial cells and the IGF system. *Journal of Molecular Endocrinology*, 54(1), R1–R13. <https://doi.org/10.1530/JME-14-0215>
- Baggio, L. L., Yusta, B., Mulvihill, E. E., Cao, X., Streutker, C. J., Butany, J., ... Drucker, D. J. (2018). GLP-1 Receptor Expression Within the Human Heart. *Endocrinology*, 159(4), 1570–1584. <https://doi.org/10.1210/en.2018-00004>
- Balaji, S., Han, N., Moles, C., Shaaban, A. F., Bollyky, P. L., Crombleholme, T. M., & Keswani, S. G. (2015). Angiopoietin-1 improves endothelial progenitor cell-dependent neovascularization in diabetic wounds. *Surgery*, 158(3), 846–856. <https://doi.org/10.1016/j.surg.2015.06.034>

- Boudina, S., & Abel, E. D. (2010). Diabetic cardiomyopathy, causes and effects. *Reviews in Endocrine & Metabolic Disorders*, 11(1), 31–39. <https://doi.org/10.1007/s11154-010-9131-7>
- Buhl, S. N., & Jackson, K. Y. (1978). Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 degrees C. *Clinical Chemistry*, 24(5), 828–831.
- Bullock, B. P., Heller, R. S., & Habener, J. F. (1996). Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology*, 137(7), 2968–2978. <https://doi.org/10.1210/endo.137.7.8770921>
- Cernea, S., & Raz, I. (2011). Therapy in the early stage: incretins. *Diabetes Care*, 34 Suppl 2, S264-271. <https://doi.org/10.2337/dc11-s223>
- Chagoya de Sánchez, V., Hernández-Muñoz, R., López-Barrera, F., Yañez, L., Vidrio, S., Suárez, J., ... Cruz, D. (1997). Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long-term and integrative study. *Canadian Journal of Physiology and Pharmacology*, 75(12), 1300–1311.
- Cheng, R., & Ma, J. (2015). Angiogenesis in diabetes and obesity. *Reviews in Endocrine and Metabolic Disorders*, 16(1), 67–75. <https://doi.org/10.1007/s11154-015-9310-7>
- Chua, S., Sheu, J.-J., Chen, Y.-L., Chang, L.-T., Sun, C.-K., Leu, S., ... Yip, H.-K. (2013). Sitagliptin therapy enhances the number of circulating angiogenic cells and angiogenesis—evaluations in vitro and in the rat critical limb ischemia model. *Cytotherapy*, 15(9), 1148–1163. <https://doi.org/10.1016/j.jcyt.2013.05.005>

- Dai, W., Kloner, R. A., Shi, J., Carreno, J., Korjian, S., Daaboul, Y., ... Isabelle, M. (2017). Effect of Diabetes on Myocardial Infarct and No Reflow Size in an Experimental Rat Model and Clinical Trial. *Journal of Clinical & Experimental Cardiology*, 8(12). <https://doi.org/10.4172/2155-9880.1000560>
- Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., ... Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet (London, England)*, 378(9785), 31–40. [https://doi.org/10.1016/S0140-6736\(11\)60679-X](https://doi.org/10.1016/S0140-6736(11)60679-X)
- Davis, T. M. E., Fortun, P., Mulder, J., Davis, W. A., & Bruce, D. G. (2004). Silent myocardial infarction and its prognosis in a community-based cohort of Type 2 diabetic patients: the Fremantle Diabetes Study. *Diabetologia*, 47(3), 395–399. <https://doi.org/10.1007/s00125-004-1344-4>
- Deniz, F., Kepez, A., Ay, S. A., Ergogan, O., Baskoy, K., Guncikan, M. N., ... Yonem, A. (2015). Evaluation of electrocardiographic parameters in patients with diabetes insipidus. *Wiener Klinische Wochenschrift*, 127(21–22), 871–876. <https://doi.org/10.1007/s00508-015-0874-8>
- Di Paola, R., Fusco, R., Gugliandolo, E., D'Amico, R., Campolo, M., Latteri, S., ... Cuzzocrea, S. (2018). The Antioxidant Activity of Pistachios Reduces Cardiac Tissue Injury of Acute Ischemia/Reperfusion (I/R) in Diabetic Streptozotocin (STZ)-Induced Hyperglycaemic Rats. *Frontiers in Pharmacology*, 9, 51. <https://doi.org/10.3389/fphar.2018.00051>

- Donath, M. Y., Sütsch, G., Yan, X. W., Piva, B., Brunner, H. P., Glatz, Y., ... Kiowski, W. (1998). Acute cardiovascular effects of insulin-like growth factor I in patients with chronic heart failure. *The Journal of Clinical Endocrinology and Metabolism*, 83(9), 3177–3183. <https://doi.org/10.1210/jcem.83.9.5122>
- Duffy, A. M., Bouchier-Hayes, D. J., & Harmey, J. H. (2013). *Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF*. Landes Bioscience. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK6482/>
- Einarson, T. R., Acs, A., Ludwig, C., & Panton, U. H. (2018). Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovascular Diabetology*, 17(1), 83. <https://doi.org/10.1186/s12933-018-0728-6>
- Ferrara, N. (2004). Vascular Endothelial Growth Factor: Basic Science and Clinical Progress. *Endocrine Reviews*, 25(4), 581–611. <https://doi.org/10.1210/er.2003-0027>
- Field, B. C. T., Chaudhri, O. B., & Bloom, S. R. (2009). Obesity treatment: novel peripheral targets. *British Journal of Clinical Pharmacology*, 68(6), 830–843. <https://doi.org/10.1111/j.1365-2125.2009.03522.x>
- Fiordaliso, F., Maggioni, S., Balconi, G., Schiarea, S., Corbelli, A., De Luigi, A., ... Latini, R. (2016). Effects of dipeptidyl peptidase-4 (DPP-4) inhibition on angiogenesis and hypoxic injury in type 2 diabetes. *Life Sciences*, 154, 87–95. <https://doi.org/10.1016/j.lfs.2016.03.052>
- Froesch, E. R., Zenobi, P. D., & Hussain, M. (1994). Metabolic and Therapeutic Effects of Insulin-Like Growth Factor I. *Hormone Research in Paediatrics*, 42(1–2), 66–71. <https://doi.org/10.1159/000184148>

- Gautier, J. F., Fetita, S., Sobngwi, E., & Salaün-Martin, C. (2005). Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with type 2 diabetes. *Diabetes & Metabolism*, 31(3 Pt 1), 233–242.
- Goyal, S. N., Arora, S., Sharma, A. K., Joshi, S., Ray, R., Bhatia, J., ... Arya, D. S. (2010). Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultrastructural alterations in isoproterenol-induced cardiotoxicity in rats. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 17(3–4), 227–232. <https://doi.org/10.1016/j.phymed.2009.08.009>
- Gros, R., You, X., Baggio, L. L., Kabir, M. G., Sadi, A. M., Mungrue, I. N., ... Husain, M. (2003). Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology*, 144(6), 2242–2252. <https://doi.org/10.1210/en.2003-0007>
- Haleagrahara, N., Chakravarthi, S., & Mathews, L. (2011). Insulin like growth factor-1 (IGF-1) causes overproduction of IL-8, an angiogenic cytokine and stimulates neovascularization in isoproterenol-induced myocardial infarction in rats. *International Journal of Molecular Sciences*, 12(12), 8562–8574. <https://doi.org/10.3390/ijms12128562>
- Hassan, M. Q., Akhtar, M. S., Akhtar, M., Ali, J., Haque, S. E., & Najmi, A. K. (2016). Edaravone, a potent free radical scavenger and a calcium channel blocker attenuate isoproterenol induced myocardial infarction by suppressing oxidative stress, apoptotic signaling and ultrastructural damage. *Therapeutic Advances in Cardiovascular Disease*, 10(4), 214–223. <https://doi.org/10.1177/1753944716630653>
- Herman, G. A., Stein, P. P., Thornberry, N. A., & Wagner, J. A. (2007). Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: focus on sitagliptin. *Clinical Pharmacology and Therapeutics*, 81(5), 761–767. <https://doi.org/10.1038/sj.clpt.6100167>

- Huang, C.-Y., Shih, C.-M., Tsao, N.-W., Lin, Y.-W., Huang, P.-H., Wu, S.-C., ... Lin, F.-Y. (2012). Dipeptidyl peptidase-4 inhibitor improves neovascularization by increasing circulating endothelial progenitor cells. *British Journal of Pharmacology*, 167(7), 1506–1519. <https://doi.org/10.1111/j.1476-5381.2012.02102.x>
- Huang, F., Liu, Y., Yang, X., Che, D., Qiu, K., Hammock, B. D., ... Huang, H. (2017). Shexiang Baoxin pills promotes angiogenesis in myocardial infarction rats via up-regulation of 20-HETE-mediated endothelial progenitor cells mobilization. *Atherosclerosis*, 263, 184–191. <https://doi.org/10.1016/j.atherosclerosis.2017.06.012>
- Inda, A. M., Andrini, L. B., García, M. N., García, A. L., Fernández Blanco, A., Furnus, C. C., ... Errecalde, A. L. (2007). Evaluation of angiogenesis with the expression of VEGF and CD34 in human non-small cell lung cancer. *Journal of Experimental & Clinical Cancer Research: CR*, 26(3), 375–378.
- Johnston, A. P. W., Campbell, J. E., Found, J. G., Riddell, M. C., & Hawke, T. J. (2007). Streptozotocin induces G₂ arrest in skeletal muscle myoblasts and impairs muscle growth in vivo. *American Journal of Physiology-Cell Physiology*, 292(3), C1033–C1040. <https://doi.org/10.1152/ajpcell.00338.2006>
- Kassahun, A., Gashe, F., Mulisa, E., & Rike, W. (2016). Nonadherence and factors affecting adherence of diabetic patients to anti-diabetic medication in Assela General Hospital, Oromia Region, Ethiopia. *Journal of Pharmacy and Bioallied Sciences*, 8(2), 124. <https://doi.org/10.4103/0975-7406.171696>
- Khodeer, D. M., Zaitone, S. A., Farag, N. E., & Moustafa, Y. M. (2016). Cardioprotective effect of pioglitazone in diabetic and non-diabetic rats subjected to acute myocardial infarction involves suppression of AGE-RAGE axis and inhibition of apoptosis. *Canadian Journal*

of Physiology and Pharmacology, 94(5), 463–476. <https://doi.org/10.1139/cjpp-2015-0135>

- Kim, D., Wang, L., Beconi, M., Eiermann, G. J., Fisher, M. H., He, H., ... Weber, A. E. (2005). (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Journal of Medicinal Chemistry*, 48(1), 141–151. <https://doi.org/10.1021/jm0493156>
- Kittnar, O. (2015). Electrocardiographic changes in diabetes mellitus. *Physiological Research*, 64 Suppl 5, S559-566.
- Kolluru, G. K., Bir, S. C., & Kevil, C. G. (2012). Endothelial Dysfunction and Diabetes: Effects on Angiogenesis, Vascular Remodeling, and Wound Healing [Research article]. <https://doi.org/10.1155/2012/918267>
- Kuo, M.-C., Patschan, D., Patschan, S., Cohen-Gould, L., Park, H.-C., Ni, J., ... Goligorsky, M. S. (2008). Ischemia-Induced Exocytosis of Weibel-Palade Bodies Mobilizes Stem Cells. *Journal of the American Society of Nephrology*, 19(12), 2321–2330. <https://doi.org/10.1681/ASN.2007111200>
- Lee, S.-B., Lee, W. S., Shin, J.-S., Jang, D. S., & Lee, K. T. (2017). Xanthotoxin suppresses LPS-induced expression of iNOS, COX-2, TNF- α , and IL-6 via AP-1, NF- κ B, and JAK-STAT inactivation in RAW 264.7 macrophages. *International Immunopharmacology*, 49, 21–29. <https://doi.org/10.1016/j.intimp.2017.05.021>
- Lee, S.-H., Kwak, C.-H., Lee, S.-K., Ha, S.-H., Park, J., Chung, T.-W., ... Kim, C.-H. (2016). Anti-Inflammatory Effect of Ascochlorin in LPS-Stimulated RAW 264.7 Macrophage Cells Is Accompanied With the Down-Regulation of iNOS, COX-2 and Proinflammatory

- Cytokines Through NF- κ B, ERK1/2, and p38 Signaling Pathway. *Journal of Cellular Biochemistry*, 117(4), 978–987. <https://doi.org/10.1002/jcb.25383>
- Lee, T.-M., Chen, W.-T., & Chang, N.-C. (2016). Sitagliptin decreases ventricular arrhythmias by attenuated glucose-dependent insulintropic polypeptide (GIP)-dependent resistin signalling in infarcted rats. *Bioscience Reports*, 36(2), e00307–e00307. <https://doi.org/10.1042/BSR20150139>
- Li, X., Zhou, R., Zheng, P., Yan, L., Wu, Y., Xiao, X., & Dai, G. (2010). Cardioprotective effect of matrine on isoproterenol-induced cardiotoxicity in rats. *The Journal of Pharmacy and Pharmacology*, 62(4), 514–520. <https://doi.org/10.1211/jpp.62.04.0015>
- Lin, C.-H., & Lin, C.-C. (2016). Sitagliptin attenuates inflammatory responses in lipopolysaccharide-stimulated cardiomyocytes via nuclear factor- κ B pathway inhibition. *Experimental and Therapeutic Medicine*, 11(6), 2609–2615. <https://doi.org/10.3892/etm.2016.3255>
- Lin, S., Zhang, Q., Shao, X., Zhang, T., Xue, C., Shi, S., ... Lin, Y. (2017). IGF-1 promotes angiogenesis in endothelial cells/adipose-derived stem cells co-culture system with activation of PI3K/Akt signal pathway. *Cell Proliferation*, 50(6), e12390. <https://doi.org/10.1111/cpr.12390>
- Liu, Y.-S., Huang, Z.-W., Wang, L., Liu, X.-X., Wang, Y.-M., Zhang, Y., & Zhang, M. (2015). Sitagliptin alleviated myocardial remodeling of the left ventricle and improved cardiac diastolic dysfunction in diabetic rats. *Journal of Pharmacological Sciences*, 127(3), 260–274. <https://doi.org/10.1016/j.jphs.2014.12.007>
- Mitsos, S., Katsanos, K., Koletsis, E., Kagadis, G. C., Anastasiou, N., Diamantopoulos, A., ... Dougenis, D. (2012). Therapeutic angiogenesis for myocardial ischemia revisited: basic

biological concepts and focus on latest clinical trials. *Angiogenesis*, 15(1), 1–22.

<https://doi.org/10.1007/s10456-011-9240-2>

Moon, H. E., Byun, K., Park, H. W., Kim, J. H., Hur, J., Park, J. S., ... Paek, S. H. (2015).

COMP-Ang1 Potentiates EPC Treatment of Ischemic Brain Injury by Enhancing Angiogenesis Through Activating AKT-mTOR Pathway and Promoting Vascular Migration Through Activating Tie2-FAK Pathway. *Experimental Neurobiology*, 24(1), 55. <https://doi.org/10.5607/en.2015.24.1.55>

Mortensen, K., Christensen, L. L., Holst, J. J., & Orskov, C. (2003). GLP-1 and GIP are

colocalized in a subset of endocrine cells in the small intestine. *Regulatory Peptides*, 114(2–3), 189–196.

Neufeld, G., Cohen, T., Gengrinovitch, S., & Poltorak, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *The FASEB Journal*, 13(1), 9–22.

<https://doi.org/10.1096/fasebj.13.1.9>

Nikolaidis, L. A., Mankad, S., Sokos, G. G., Miske, G., Shah, A., Elahi, D., & Shannon, R. P.

(2004). Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation*, 109(8), 962–965. <https://doi.org/10.1161/01.CIR.0000120505.91348.58>

Nyström, T., Gutniak, M. K., Zhang, Q., Zhang, F., Holst, J. J., Ahrén, B., & Sjöholm, A. (2004).

Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *American Journal of Physiology. Endocrinology and Metabolism*, 287(6), E1209–1215. <https://doi.org/10.1152/ajpendo.00237.2004>

Patry, C., Stamm, D., Betzen, C., Tönshoff, B., Yard, B. A., Beck, G. C., & Rafat, N. (2018).

CXCR-4 expression by circulating endothelial progenitor cells and SDF-1 serum levels

are elevated in septic patients. *Journal of Inflammation (London, England)*, 15.

<https://doi.org/10.1186/s12950-018-0186-7>

Piepoli, M. F., Hoes, A. W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L., ... Gale, C.

(2016). 2016 European Guidelines on cardiovascular disease prevention in clinical practice The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart Journal*, 37(29), 2315–2381.

<https://doi.org/10.1093/eurheartj/ehw106>

Povsic, T. J., Zavodni, K. L., Vainorius, E., Kherani, J. F., Goldschmidt-Clermont, P. J., &

Peterson, E. D. (2009). Common endothelial progenitor cell assays identify discrete endothelial progenitor cell populations. *American Heart Journal*, 157(2), 335–344.

<https://doi.org/10.1016/j.ahj.2008.10.010>

Rae, P. C., Kelly, R. D., Egginton, S., & St John, J. C. (2011). Angiogenic potential of endothelial progenitor cells and embryonic stem cells. *Vascular Cell*, 3(1), 11.

<https://doi.org/10.1186/2045-824X-3-11>

Read, P. A., Khan, F. Z., Heck, P. M., Hoole, S. P., & Dutka, D. P. (2010). DPP-4 inhibition by sitagliptin improves the myocardial response to dobutamine stress and mitigates stunning

in a pilot study of patients with coronary artery disease. *Circulation. Cardiovascular Imaging*, 3(2), 195–201. <https://doi.org/10.1161/CIRCIMAGING.109.899377>

Samikannu, B., Chen, C., Lingwal, N., Padmasekar, M., Engel, F. B., & Linn, T. (2013).

Dipeptidyl Peptidase IV Inhibition Activates CREB and Improves Islet Vascularization

through VEGF-A/VEGFR-2 Signaling Pathway. *PLoS ONE*, 8(12), e82639.

<https://doi.org/10.1371/journal.pone.0082639>

Sauvé, M., Ban, K., Momen, M. A., Zhou, Y.-Q., Henkelman, R. M., Husain, M., & Drucker, D. J. (2010). Genetic deletion or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes after myocardial infarction in mice. *Diabetes*, 59(4), 1063–1073. <https://doi.org/10.2337/db09-0955>

Scirica, B. M., Bhatt, D. L., Braunwald, E., Steg, P. G., Davidson, J., Hirshberg, B., ... Raz, I. (2013). Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. *New England Journal of Medicine*, 369(14), 1317–1326. <https://doi.org/10.1056/NEJMoa1307684>

Siavashi, V., Sariri, R., Nassiri, S. M., Esmaeilvand, M., Asadian, S., Cheraghi, H., ... Rahbarghazi, R. (2016). Angiogenic activity of endothelial progenitor cells through angiopoietin-1 and angiopoietin-2. *Animal Cells and Systems*, 20(3), 118–129. <https://doi.org/10.1080/19768354.2016.1189961>

Skovsø, S. (2014). Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *Journal of Diabetes Investigation*, 5(4), 349–358. <https://doi.org/10.1111/jdi.12235>

Vaessen, N., Heutink, P., Janssen, J. A., Witteman, J. C., Testers, L., Hofman, A., ... van Duijn, C. M. (2001). A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes*, 50(3), 637–642.

Wilson, P. W., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., & Kannel, W. B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation*, 97(18), 1837–1847.

- Wu, F., Yu, B., Zhang, X., & Zhang, Y. (2017). Cardioprotective effect of Notch signaling on the development of myocardial infarction complicated by diabetes mellitus. *Experimental and Therapeutic Medicine*, 14(4), 3447–3454. <https://doi.org/10.3892/etm.2017.4932>
- Würzburg, U., Hennrich, N., Lang, H., Prellwitz, W., Neumeier, D., & Knedel, M. (1976). Bestimmung der Aktivität von Creatinkinase MB im Serum unter Verwendung inhibierender Antikörper. *Klinische Wochenschrift*, 54(8), 357–360. <https://doi.org/10.1007/BF01469790>
- Yang, J., Ba, T., Chen, L., Shan, C., Zheng, M., Wang, Y., ... Chang, B. (2016). Effects of metformin and sitagliptin on glycolipid metabolism in type 2 diabetic rats on different diets. *Archives of Medical Science : AMS*, 12(2), 233–242. <https://doi.org/10.5114/aoms.2016.59249>
- Yousefi, K., Soraya, H., Fathiazad, F., Khorrami, A., Hamedeyazdan, S., Maleki-Dizaji, N., & Garjani, A. (2013). Cardioprotective effect of methanolic extract of *Marrubium vulgare* L. on isoproterenol-induced acute myocardial infarction in rats. *Indian Journal of Experimental Biology*, 51(8), 653–660.
- Zander, M., Madsbad, S., Madsen, J. L., & Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet (London, England)*, 359(9309), 824–830. [https://doi.org/10.1016/S0140-6736\(02\)07952-7](https://doi.org/10.1016/S0140-6736(02)07952-7)
- Zhang, G.-X., Kimura, S., Nishiyama, A., Shokoji, T., Rahman, M., Yao, L., ... Abe, Y. (2005). Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovascular Research*, 65(1), 230–238. <https://doi.org/10.1016/j.cardiores.2004.08.013>

Table 1. Effect of treatment with sitagliptin (5 or 10 mg/kg) on left ventricular body weight to total body weight and heart weight ratios in diabetic rats with acute myocardial infarction.

Groups	Baseline BW (g)	Final BW (g)	% change BWt	LV wet to BWt ratio(g/g) x10 ⁻³	HtW wet to BWt ratio (g/g) x10 ⁻³
Vehicle	113 ± 1.56	123 ± 3.7	8.84 ± 2.2	0.9 ± 0.1	4.1 ± 0.1
Diabetic	104.3 ± 1.3	142.1 ± 2.9	36.24 ± 1.7*	1.9 ± 0.1*	5 ± 0.2*
Diabetic + MI	105 ± 1.5	142.6 ± .87	35.8 ± 1.4*	2 ± 0.1*	5 ± 0.5*
Diabetic + MI+ sitagliptin (5 mg/kg)	146 ± 1.4	171.4 ± 7	17.3 ± .9*&\$	1.2 ± 0.1&\$	3.2 ± 0.2*&\$
Diabetic + MI + sitagliptin (10 mg/kg)	147.5 ± 1.4	181.6 ± 7	23.1 ± 1.3*&\$%	0.7 ± 9.2&\$	3 ± 0.1*&\$

Percent change in BWt was calculated using a formula: % change BWt = [(final BWt- baseline BWt)/ baseline BWt] x 100. Baseline BWt: was recorded at the first week and final BWt was recorded at the end of week 5. Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni's post hoc test at $P < 0.05$. *Significantly different from vehicle group, &Significantly different from diabetic group, \$Significantly different from diabetic+MI group, %Significantly different from diabetic+MI +sitagliptin (5 mg/kg) group.

Table 2. Effect treatment with sitagliptin (5 or 10 mg/kg) on lactate dehydrogenase and creatine kinase-MB activities in diabetic rats with acute myocardial infarction.

Groups	CK-MB (U/L)	LDH (U/L)
Vehicle	425.5±41.9	233.3±18.8
Diabetic	443.6±31.6	365.7±23.6
Diabetic + MI	698.96±34.17*&	874.26±56.08*&
Diabetic + MI + sitagliptin (5 mg/kg)	566.63±25.57*&	778.82±81.7*&
Diabetic + MI + sitagliptin (10 mg/kg)	476.61±28.84 [§]	524.02±131.65 [§]

Data are mean±SEM and analyzed using one-way ANOVA followed by Bonferroni's *post-hoc* test at $P<0.05$.

* different from vehicle group.

& different from diabetic group.

[§] different from diabetic + MI group.

% different from diabetic + MI + sitagliptin (5 mg/kg) group.

Draft

Figures captions:

FIGURE 1: Effect of sitagliptin (5 or 10 mg/kg) on fasting blood glucose. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 2. a) The electrocardiographic effect of sitagliptin in different two doses on isoproterenol-induced MI in diabetic groups. **b)** Effect of sitagliptin on isoproterenol-induced changes in heart rate, QRS, QT, QTc, ST intervals and R amplitude in the diabetic groups. bpm: beat per minute, ms: millisecond. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 3: Effect of sitagliptin (5 or 10 mg/kg) on heart tissue homogenate level of IGF. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 4: Effect of sitagliptin (5 or 10 mg/kg) on heart tissue homogenate level of IL 6. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 5: Effect of sitagliptin (5 or 10 mg/kg) on heart tissue homogenate level of COX 2. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 6: Histopathology images for sections from the heart of the experimental diabetic groups stained with hematoxylin and eosin (x 40). A: Section from the heart of saline treated rats (vehicle) shows normal appearance. B: In diabetic group, image shows moderate histopathological changes. C: Diabetic+MI group represents group at which rats myocardial tissue submitted to infarction, increase in number of neutrophils, nuclei showed decreased size and condensation, tissue showed loss of striations and sarcoplasm was densely eosinophilic, with degeneration indicated by arrows. D: Diabetic+MI+sitagliptin (5 mg/kg) group shows, moderately histopathological changes. E: Diabetic+MI+sitagliptin (10 mg/kg) group shows, mild histopathological changes. MI: Myocardial infarction.

FIGURE 7. Mean rank of different pathological changes grades in the cardiac tissue samples from different groups. a: Boxplot of pathological changes grades in the cardiac tissue samples from different groups. Difference between all treatment groups assessed using Kruskal Wallis test statistic at $p < 0.001$ followed by Mann-Whitney pairwise comparisons test to evaluate difference between groups at $p < 0.001$. b: Pairwise comparisons of pathological changes grades in the cardiac tissue samples from different groups. Difference between all treatment groups assessed using Kruskal Wallis test statistic at $p < 0.05$ followed by Mann-Whitney pairwise comparisons test to evaluate difference between groups at $p < 0.05$.

FIGURE 8: a) Immunohistochemical staining for VEGF in heart tissue experimental groups (DAB and Mayer's hematoxylin x100). **b)** Optical density for VEGF immunostaining in the experimental groups. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

FIGURE 9: a) Immunohistochemical staining for CD34 in heart tissue experimental groups (DAB and Mayer's hematoxylin x100). **b)** Optical density for CD34 immunostaining in the experimental groups. Data were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed Bonferroni's post-hoc test at $P < 0.05$. *Significantly different from vehicle group. &Significantly different from diabetic group. §Significantly different from diabetic+MI. %Significantly different from diabetic+MI+sitagliptin (5 mg/kg) group, $n = 8$.

Draft

Fig. 1

123x75mm (300 x 300 DPI)

Fig. 2 a,b

1343x858mm (72 x 72 DPI)

Fig. 3

109x88mm (300 x 300 DPI)

Fig. 4

97x95mm (300 x 300 DPI)

Fig. 5

105x106mm (300 x 300 DPI)

Fig. 6

108x132mm (300 x 300 DPI)

Fig. 7

138x180mm (300 x 300 DPI)

fig. 8 a,b

139x189mm (300 x 300 DPI)

Fig. 9 a,b

140x194mm (300 x 300 DPI)