Novel potentials of dipeptidyl peptidase-4 inhibitor sitagliptin against ischemia-reperfusion (I/R) injury in normolipidemic and hyperlipidemic animals

Summary of Ph.D. Thesis

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Summary

The isolated perfused heart is a convenient and reproducible model to test cellular and metabolic mechanisms of myocardial injury, and for screening drugs or interventions for cardioprotective properties.

In our study, we isolated heart tissues of wistar rats fed with normal or three months with high-fat diet (HF) and pre-treated daily for 14 days with saline or different doses of sitagliptin (25, 50, 100 or 150 mg/kg) and assigned them into a Langendorff system through aorta cannulation, using prolonged and brief reperfusion protocols, for infarct size (IS) and biochemical measurements respectively.

The 50 mg dose of sitagliptin exhibited a significant decrease in infarct size in both normal and high-fat diet animals. Gliptins are well known for their anti-hyperglycaemic and incretin homeostatic properties, namely the glucagon-like peptide-1 (GLP-1). Sitagliptin treatment showed a significant decrease in DPP-4 activity and increase in GLP-1level in normal animals but not in hyperlipidemic ones.

We extended our measurements to cNOS activity, and endothelial nitric oxide synthase (e-NOS) protein expression, followed by transient receptor potential vaniloid-1 (TRPV-1) level and transient receptor potential canonical- 1 (TRPC-1) protein expression. A significant upregulation in NOS activity was the case in animal's groups of both diet conditions. e-NOS expression, TRPV-1 level and TRPC-1 expression increased significantly in normal diet animals, however, in high-fat (HF) diet animals this upregulatory effect was abolished.

Transient receptor potential vanilloid type-1 (TRPV-1) is an upstream regulator of calcitonin gene-related peptide (CGRP), and TRPV-1 stimulation promotes the release of CGRP, mediating cardioprotection. Obtained results from CGRP measurement displayed a significant increase in normal animals as well as in animals kept on high-fat diet, after treatment with sitagliptin.

We invesitgated the effect of nitric oxide synthase (NOS) inhibition on infarct size in animal groups from both diets, and TRPV-1 inhibitory effect in normal animals only. The cardioprotective effect of sitagliptin mediated by NOS was lost in both diet conditions. Similarly, the inhibitory effect of TRPV-1 showed an increase in infarct size, and blockage of sitagliptin-TRPV-1 mediated cardioprotective action.

Introduction

The incidence of cardiovascular disorders continues to grow across worldwide, contributing to the largest rate of mortality and morbidity each year, including ischemic heart disease . Ischemia-reperfusion (I/R) injury occurs when circulation is abruptly restored following prolonged ischemia and characterized by high levels of calcium and tissue neutrophil accumulationn causing cellular damage, and production of reactive oxygen species (ROS) during reperfusion, triggering I/R injury

Reperfusion is necessary for the restoration of epicardial and microvascular blood flow and the normal physiology of the heart, avoiding further damage to the mayocardium , however, exposing heart tissues to abrupt reperfusion, can lead to further myocardial damage, and subsequently cell death .

Hyperlipidemia is considered a significant reason and major contributor in the development of ischemic heart disease and myocardial infarction (MI), responsible for lipids deposition in athermanous lesions and primary endothelail injury, and long-term high-fat diet (HFD) consumption can increase myocardial infarct size following an ischemia-reperfusion insult.

Previously experimented pharmacological drugs under normolipidemic and hyperlipidemic conditions failed to make their way into clinical treatments. Therefore, finding new protective agents and molecular targets during I/R that can be exploited therapeutically in the aim of decreasing the incidence of cardiovascular events and limiting the extent of infarction is urgently needed.

Dipeptidyl Peptidase-4 (DPP-4) is a widely expressed glycoprotein peptidase that exhibits catalytic degradation of incretins such as glucagon-like-peptide-1 (GLP-1) . A number of pharmaceutical products including dipeptidyl peptidase-4 (DPP-4) inhibitors such as sitagliptin are oral anti-diabetic drugs that inhibit the DPP-4 enzyme, avoid the degradation of incretins , and result in its prolonged action . Activation of GLP-1 by DPP4-4 inhibitors, limits myocardial infarct size (IS) and protects cardiomyocytes from cell death.

Nitric oxide synthase (NOS) is an enzyme that catalyzes the production of nitric oxide (NO) from L-arginine, and exhibits cardioprotective effects by decreasing infarct size in acute

myocardial infarction . Endothelial NOS (e-NOS) is highly abundant in endothelail cells, as well as in cardiomyocytes. Up-regulation of GLP-1 can restore vascular NO bioavailability by the up-regulation of e-NOS, and blocking DPP-4 activity plays a role in the modulation of NOS enzymes

Transient receptor potential (TRP) channels, namely the canonical (TRPC) and vanilloid (TRPV) isoforms are the most commonly localized and essential Ca^{2+}-permeable channels in vascular endothelial cells, ventricles, coronary blood vessels and sensory nerves innervating the heart.

TRPV-1 are localized in the sensory nerves that surrounds the cardiovascular structure and in cardiomyocytes. The latter functions upon a chemical or physical stimuli like capsaicin (CAP), proton and heat, releasing sensory neuropeptides functioning as vasodilators, like calcitonin gene-related peptide (CGRP), mediating cardioprotection and regulating the cardiac function . Activation of endothelial nitric oxide synthase (e-NOS) mediates the endothelium-dependent vasodilatory actions induced by CGRP, and since e-NOS is Ca^{2+}/calmodulin-dependent, its activity may be modulated by cytosolic Ca^{2+} levels . Although TRPC and TRPV channels play a fundamental role in mediating ischemia-reperfusion injury and regulating cardioprotective signaling; however, the activation mechanisms underlying both types of channels are still unknown, and further investigations are needed to testify the significance and contribution of CGRP in cardioprotection.

Aims

Due to the pleiotropic cardioprotective effect of gliptins as anti-diabetic drugs, we aimed to study the effect sitagliptin treatment on myocardial I/R injury in normal and high-fat diet animals, using an *ex-vivo* model.

We hypothesized that:

1. Sitagliptin treatment can decrease the infarct size in both diet conditions
2. Protective effect of sitagliptin in normal and high-fat diet animals can be mediated by:
    i. Nitric oxide synthase (NOS)
    ii. Transient receptor potential channels (TRPC, TRPV)

iii. Calcitonin gene-related peptide (CGRP)

3. NOS and TRPV inhibition can block the cardioprotective effect of sitagliptin

Materials and Methods

In this study, we used an isolated *ex-vivo* perfused heart model to study ischemia-reperfusion (I/R) injury. This model is ideal for screening drugs or interventions for protective properties, because heart tissue is studied independently of circulating factors or neuroendocrine inputs from other organs but retains the function, composition, and architecture of the intact heart.

Animals and experimental design

Wistar rats were assigned into 2 different diet conditions: fed either with standard rat chow only or mixed with fats (High fat= HF) for 12 weeks to induce hyperlipidemia.
Animals were then divided into 4 different experiments:

Experiment 1. To determine the effective dose of Sitagliptin (Sitg), animals from both diets were randomly divided into 5 groups: (Control (Saline), Sitg (25 mg), Sitg (50 mg), Sitg (100 mg) and Sitg (150 mg), n=8-16). Daily oral administration of different drug doses or its vehicle (Saline) lasted for two weeks. Animals were then anesthetized, heart tissues were excised, immediately placed in ice-cold saline, mounted and ligated through the aorta into the cannula of a Langendorff Apparatus System, and perfused with 37 °C Krebs buffer. Hearts were exposed to 10 min perfusion, 45 min prolonged regional ischemia by LAD coronary artery occlusion, and 120 min reperfusion. LAD was then re-ligated, and stained with Evans blue via the aortic root, to determine the AAR. Tissues were stored at -20 ^{o}C for TTC staining.

Experiment 2. For the purpose of *in vitro* laboratory measurements, another set of experiments was carried out on 2 animal groups (Control (Saline) and Sitg (50 mg/kg/day), n=10), from both diets. Oral treatment lasted for 2 weeks. Same whole-heart preparation procedure as in experiment 1 was carried out. Hearts were exposed to 10 min perfusion, 45 min prolonged regional ischemia, and 10 min brief reperfusion. Tissues were clamped and stored at -80 ^{o}C for biochemical analyses.

Experiment 3. To check the involvement of NOS in Sitg- mediated cardioprotection, four different animal groups (Control (Saline), Sitg (50 mg), Control (Saline) + L-NAME, and Sitg

(50 mg) + L-NAME, n=10-12) were studied in animals from both diets. Oral treatment lasted for 2 weeks, with intraperitoneal (i.p) injection of NOS inhibitor (L-NAME). Same heart preparation procedure and I/R injury protocol were applied as in Experiment 1. Tissues were stored at -20 ^{o}C for TTC staining and IS measurement.

Experiment 4. To evaluate the inhibitory effect of TRPV-1, four groups only from normolipidemic animals (Control (Saline) + DMSO, Sitg (50 mg) + DMSO, Control (Saline) + CAP, and Sitg (50 mg) + CAP, n= 5-8) received 2 weeks' oral treatments, and intraperitoneal injections with DMSO or TRPV-1 inhibitor (Capsazepine (CAP)). Same heart preparation procedure, I/R injury protocol, and tissue- staining procedure were performed similarly as in Experiment 1.

Tissue staining and infarct size measurement

Frozen tissues were transversely sectioned into 5-6 slices, and incubated in TTC, fixed in formalin, and then placed in phosphate buffer. Sections were mounted on glass slides, images were captured with a digital camera, and analysed with an ImageJ software.

Serum cholesterol and triglyceride measurements

Blood samples only from normal and high-fat diet animals were centrifuged, serum collected, cholesterol (Chol) and triglyceride (TG) levels were measured using specific reagent kits. Chol and TG serum concentrations were quantitatively determined based on enzymatic colorimetric method (phenol + aminophenazone -PAP) at 490-550 nm. Results of both measurements were expressed in mmol/l.

Liver cholesterol and triglyceride measurements

Liver tissues from high-fat diet animals were homogenized in ice-cold phosphate buffer saline by Ultra-Turrax T25 (13,500/s). Supernatants were collected, and same chol and TG kits (Diagnosticum Zrt) were used. Obtained results were expressed in (mmol/l).

DPP-4 activity

DPP-4 activity was measured from heart tissues of normal and high-fat diet animals, using specific DPP-4 activity assay kit (Sigma-Aldrich). Tissues were homogenized in ice-cold assay Buffer, centrifuged at 13,000 x g, for 10 min at 4°C, Standard and sample fluorescence intensities from collected supernatants were at 360-460 nm in 96-well black plates specific for fluorescence assays, using fluorescence multi-well plate reader (Fluorometer). Results are expressed as microunit/ml.

Nitric oxide synthase (NOS) activity

NOS activity was measured in heart tissues from animals fed with both diets, by quantifying the conversion of [^{14}C]-labeled L-arginine to citrulline. Heart tissues were homogenized in ice-cold special homogenization buffer of pH 7.4. Samples were centrifuged for 30 min, at 20000 x g, and 4°C. Samples were then incubated for 10 min at 37°C with an assay buffer. The reaction was terminated by addition of ice-cold DOWEX prepared in distilled water. The mixture was re-suspended by adding ice-cold distilled water, supernatants were removed and radioactivity was determined by scintillation counting. The Ca^{2+} dependence of the NOS activity was determined by addition of ethylene glycol-bis tetraacetic acid (EGTA). NOS activity was confirmed by inhibition with Nω-nitro-L-arginine methyl ester (L-NNA). The i-NOS level was defined as the extent of citrulline formation that was inhibited by L-NNA, but not by EGTA. The cNOS activity was calculated from the difference between the extent of citrulline formation inhibited by EGTA and the total activity. This activity is referred to as cNOS, and expressed as pmol/min/mg protein.

ELISA measurements (GLP-1, TRPV-1 and CGRP)

A double-antibody sandwich ELISA kits specific for rat GLP-1, TRPV-1 and CGRP (SunRed Biotechnology) were used for measurements form heart tissues in both animal diets, using same homogenization buffer (PBS), homogenization and centrifugation procedures. Parameters were measured at 450 nm, and results were expressed in ng/ml for GLP-1 and TRPV-1, and ng/L for CGRP.

Calcium (Ca^{2+}) content

A Colorimetric Calcium Detection Assay Kit (Abcam) was used to determine the calcium (Ca^{2+}) concentration in heart tissues of animals from both diets. Samples were homogenized on ice-cold PBS buffer, and centrifuged at a maximum speed for 2-5 min. Supernatants were collected, and optical densities (OD) were detected at 575 nm. Results were expressed in ng/mg protein.

TRPC-1 and e-NOS and DPP-4 (CD26) protein expression by western blotting normalized to β-actin

Measured heart tissues from both diet groups were homogenized at 13,500/s in ice-cold radio RIPA buffer, and Homo-buffer, for TRPC-1 and e-NOS proteins respectively. Homogenates were centrifuged (10-15 min, 12000 rpm, 4°C). Proteins were resolved on 8 % and 10 % sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and transferred to nitrocellulose membranes. Blots were probed overnight (4 ^{o}C, and 1 % milk), with anti-TRPC-1 rabbit primary antibody (1:500), anti- e-NOS mouse primary antibody (1:250), anti-CD26 rabbit primary antibody (1:500, (ab129060), and anti-beta actin mouse primary antibody (1 % BSA, 1:4000, (ab 8226). Membranes were then incubated for 1 h at room temperature with secondary anti-rabbit (1:1000, (sc-2370)), secondary anti-mouse (1:5000, (sc-A9044)), secondary anti-rabbit (1:5000, (sc-2370)), and secondary anti-mouse (1:2000, (sc-A9044)) antibodies conjugated with horseradish peroxidase (HRP) enzyme, for TRPC-1, e-NOS, CD26 (DPP4), and β-actin, respectively. Signals were developed by using enhanced chemiluminescent substrate for detection of HRP and exposed to Hyperfilm. Protein bands densities were analysed using the Image Quant Software.

Protein determination

Aliquots from diluted samples (15- or 25-fold with distilled water) were mixed with distilled water, and Bradford reagent. After mixing and 10 min incubation, samples were assayed spectrophotometrically at 595 nm (Benchmark Microplate Reader; Bio-Rad, Budapest, Hungary). Protein levels were expressed as mg protein/ml.

Statistical analysis

All data were shown as mean ± SEM. Statistical comparisons were performed with Student's two-tailed unpaired *t* test, one-way ANOVA multiple comparison test (Bonferroni), and two-way ANOVA when necessary. Differences were considered significant when P- values were less than 0.05 ($P < 0.05$).

Results

Evaluation of sitagliptin effect on infarct size

In sitagliptin (50 mg/kg/day) treated animal groups, the infarct size decreased significantly compared to the controls (saline), in both normal and high-fat diet animals. Although a decrease in infarct size was also observed in other doses, but results were not significant.

Serum cholesterol and triglyceride

Cholesterol and triglyceride measurements from serum samples revealed a significant increase in high-fat control animals compared to normal control group, however, no significant change was observed in groups treated with sitagliptin.

Liver cholesterol and triglycerides concentration in high-fat diet animals

Measurements from liver homogenates showed a significant decrease in cholesterol level in sitagliptin (50 mg)- treated group compared to control (saline), while no significant difference was observed in comparison with absolute controls. Liver triglyceride significantly increased in high-fat control group compared to absolute controls, but no significant change was observed in the group treated with sitagliptin.

Heart tissue DPP-4 activity and GLP-1 level

Both, DPP-4 activity and GLP-1 level were significantly upregulated after sitagliptin (50 mg) treatment in normolipidemic animals, while not in case of hyperlipidemia.

DPP-4 level in Heart tissues and Aorta's of high-fat diet animals

Results from heart tissues and aorta's exhibited a significant reduction in DPP-4 level in sitgliptin (50 mg) treated group compared to control (Saline).

DPP-4 (CD26) protein expression in high-fat diet animals

No significant difference in DPP-4 (CD26) expression was observed in animal groups treated with sitagliptin (50 mg) dose, compared to the control.

TRPV-1 and CGRP levels in heart tissues

Sitagliptin (50 mg) treatment caused a significant increase in TRPV-1 level in normal animals, but not in high-fat diet ones, in comparison with the controls. Interestingly, CGRP levels increased significantly in animal groups treated with sitagliptin (50 mg) and in both diet conditions.

Cardiac calcium content

To determine whether the ischemic cardiac calcium concentration was affected by sitagliptin treatment, calcium level was measured calorimetrically, and obtained findings indicated an increase in calcium content in heart tissues assigned to drug therapy in normal and high-fat diet animals.

Abundancy of TRPC-1 protein expression

Animal group treated with sitagliptin (50 mg) dose showed a 3- fold higher level of TRPC-1 expression in normolipidemic animals, while it was not the case in high-fat diet animals, and no significant change was observed.

cNOS activity and e-NOS protein expression

Two weeks daily oral treatment with Sitagliptin (50 mg) showed a significant increase in Nos activity in normal and high-fat diet animals, compared to the controls, however, results of e-NOS expression as determined by western blot showed an increased level of expression in sitagliptin (50 mg) treated animal group, in normal animals but not in high-fat diet groups.

Inhibitory effect of L-NAME on infarct size in normal and high-fat diet animals

The infarct size- limiting effect of sitagliptin (50 mg) was abolished in animal groups co-treated with sitagliptin (50 mg) and NOS specific inhibitor (L-NAME) compared to animal groups treated groups with sitagliptin (50 mg) only, in normolipidemic and hyperlipidemic animals. An increase in size of infarction was observed in these groups.

Inhibitory effect of capsazepine on infarct size in normolipidemic animals

The infarct size- limiting effect of sitagliptin (50 mg) in normal animals was abolished upon its co-treatment with TRPV-1 specific inhibitor (capsazepine), compared to the animal groups treated with sitagliptin (50 mg) alone. The size of infarction increased significantly in these groups.

Discussion

In the present study, treatment with sitagliptin (50 mg) for 2 weeks successfully (i) attenuated infarct size (IS), increased NOS activity, CGRP level, and calcium content in both diets, (ii) reduced DPP-4 activity and DPP-4 level in normal and high-fat diet animals respectively. The upregulation of GLP-1 and TRPV-1 levels, e-NOS and TRPC-1 proteins expression in normolipidemic groups, were abolished under hyperlipidemic condition. Taking into account the results of the ineffective doses of sitagliptin, this drug can be considered clinical relevant for the treatment of ischemic diseases at a further level, after clarifying the molecular mechanisms underlying these doses. Although sitagliptin therapy seemed to be cardioprotective in normolipidemic animals and in some part in animals kept on high-fat diet, this drug may lose its efficacy in hyperlipidemic condition, when patients suffer from hyperlipidemia as a cardiovascular co-morbidity and risk factor.

When the circulation is abruptly restored after a prolonged myocardial ischemia, this can lead to cardiomyocyte damage, which is commonly referred to myocardial I/R injury, triggered by neutrophil accumulation, causing ROS production and cellular damage. In the present study, 45 min of regional ischemia and 120 min of reperfusion in sustained I/R injury, revealed a significant percentage of infarction (50-60 %). Accordingly, developing cytoprotective strategies in the frame of limiting myocardial infarction and maintaining a proper blood flow to the ischemic myocardial region is one of the main focuses of preclinical and clinical research.

Sitagliptin 50 mg showed a significant decrease in infarct size into 22 % and increase in cNOS activity in normal diet animals, as well as after high-fat diet enriched food. This DPP-4 inhibitor (DPP-4i)- mediated cardioprotective effect was lost after NOS-inhibition by L-NAME, under both diet conditions.

Clinical investigations and experimental animal studies suggested that incretins, namely GLP-1 can exhibit cardioprotective potentials following myocardial ischemia (MI). DPP-4 is abundantly expressed in the cardiovascular system and endothelial cells, and blocking its activity can have advantageous cardiovascular outcomes.

DPP-4 inhibitors drugs were extensively studied in healthy animal models as a remedy against cardiovascular disorders, while their interventional mechanisms were poorly addressed in diseased animal models like hyperlipidemia. In our study, the effect of human- like hyperlipidaemia on development of myocardial infarction (MI) following coronary occlusion (Ischemia/Reperfusion- injury) was studied using the high-fat diet (HF) animals. Treatment with sitagliptin (50 mg) showed no decrease in serum cholesterol and triglyceride levels, however, a decrease in hepatic cholesterol was observed in sitagliptin treated group, compared to controls.

After 45 min of regional ischemia and 2 hrs of reperfusion, only 39 % of the area at risk (AAR) became necrotic in hyperlipidemic vs. 44 % in normolipidemic animals. High-fat diet does not seem to increase the susceptibility of the myocardium to I/R injury, with the importance of early reperfusion after acute myocardial infarction (AMI) in normal and high-fat diet conditions.

The peptide hormone GLP-1 was found to be highly abundant in the heart, exhibiting beneficial effects. Treatment with sitagliptin showed a significant decrease in DPP-4 activity in heart tissues from normolipidemic animals, and a significant decrease in DPP-4 level in heart tissues and aorta of high-fat diet groups. However, the expected increase in GLP-1 level was not the case in animals fed with high-fat diet, compared to the normal diet animals.

The deleterious consequences of I/R injury can be a major cause of endothelial dysfunction, causing a reduction in endothelial nitric oxide synthase (e-NOS) expression, and prolonged myocardial ischemia was found to decrease cNOS activity and e-NOS expression. Our findings revealed an increase in e-NOS expression in ischemic hearts pre-treated with sitagliptin in

normal animals but not in high-fat diet ones. Hypercholesterolemia was found to be associated with impaired endothelial function in coronary circulation.

The cardioprotective action of sitagliptin- mediated by NOS was confirmed by NOS inhibition, and this cardioprotective effect was abrogated when animals were co-treated with sitagliptin and NOS inhibitor (L-NAME), in both normal and high-fat diet animals.

The up-regulatory effect of TRPC and TRPV channels was found to be associated with the pathophysiology of vascular and cardiac tissues. Our results are in disagreement with these findings, showing a significant increase in TRPV-1 TRPC-1 after sitagliptin treatment. Intraperitoneal administration of TRPV-1 inhibitor (capsazepine) blocked the infarct size limiting effect of sitagliptin.

Stimulation of TRPV-1 promotes the release of calcitonin gene-related peptide (CGRP), with accumulating data reporting the advantageous role of CGRP in cardioprotection. This protective effect is in concordance with measurements from our study. The protective effect of sitagliptin mediated by TRPV/TRPC upregulation in normolipidemic animals was lost after a long-term high fat- diet consumption. CGRP increased significantly after prolonged high-fat diet regime and oral treatment with sitagliptin. To the best of our knowledge, this study was the first to show the upregulation of TRPV-1/CGRP axis in prolonged ischemia-reperfusion injury, using sitagliptin as a new targeting therapy, in normal and high-fat diet animals.

Conclusions

Sitagliptin treatment exhibited a:

- Significant decrease in infarct size in both normolipidemic and hyperlipidemic animals.
- Significant decreased in DPP-4 activity in normal animals but not in high-fat diet groups.
- Significant increase in GLP-1 level was clearly observed in normal animals only.
- Upregulation in heart NOS activity in animals from both diet conditions.
- Significant increase in e-NOS expression in normal animals, but not in high-fat diet groups.

- Significant increase in TRPV-1 level and TRPC-1 expression in animals kept on normal diet.
- Significant increase in calcitonin gene-related peptide in animal groups from both diets.

The cardioprotective effect of sitagliptin- mediated by NOS was lost in both diet conditions, using NOS inhibitor.

The cardioprotective effect of sitagliptin- mediated by TRPV-1 was also lost in normal animals, after TRPV-1 inhibition.

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Attestation of Authorship

*I, **Amin Al-awar** hereby declare that this submisson is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by athor person.*

LIST OF PUBLICATIONS (MTMT number: 10053167)

Publications related to thesis

Full papers (IF: 6.577)

1. **Al-Awar A**, Almási N, Szabó R, Takacs I, Murlasits Z, Szűcs G, Török S, Pósa A, Varga C, Kupai K. ***Novel Potentials of the DPP-4 Inhibitor Sitagliptin against Ischemia-Reperfusion (I/R) Injury in Rat Ex-Vivo Heart Model***. *Int J Mol Sci*. 2018 Oct 18;19(10). pii: E3226. doi: 10.3390/ijms19103226. **IF: 3.687**

2. **Al-Awar A**, Kupai K, Veszelka M, Szűcs G, Attieh Z, Murlasits Z, Török S, Pósa A, Varga C. ***Experimental Diabetes Mellitus in Different Animal Models***. *J Diabetes Res.* 2016;2016:9051426. doi: 10.1155/2016/9051426. **IF: 2.89**

3. **Al-awar A**, Almási N, Szabó R, Ménesi R, Szűcs G, Török S, Pósa A,Varga C, Kupai K. ***Effect of DPP-4 inhibitor Sitagliptin against Ischemia-Reperfusion (I/R) injury in hyperlipidemic animals*** (Under review in *Acta Biol Hung*). **IF: 0.439**

Publications not related to thesis

1. Kupai K, Szabó R, Veszelka M, **Al-Awar A**, Török S, Csonka A, Baráth Z, Pósa A, Varga C. ***Consequences of exercising on ischemia-reperfusion injury in type 2 diabetic Goto-Kakizaki rat hearts: role of the HO/NOS system***. *Diabetol Metab Syndr*. 2015 Oct 6;7:85. doi: 10.1186/s13098-015-0080-x. **IF: 2.413**

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