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Antioxidant Effects of Sitagliptin in a Rat Model of Intestinal Ischemia/Reperfusion Injury

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ABSTRACT

Objectives: This study aimed to assess the possible antioxidant role of sitagliptin on intestinal ischemia/reperfusion (II/R)-mediated tissue injury. **Methods:** Forty-five male *Sprague-Dawley* rats were randomly assigned into three groups: Sham group (operation without clamping), II/R group (operation with clamping) and Sitagliptin pretreated group (300 mg/kg/day; orally by gavage) for 2 weeks before II/R insult. II/R was performed by clamping the superior mesenteric artery for 30 min, followed by 60 min reperfusion after removal of clamping. At the end of the experimental period, all rats were sacrificed for biochemical assessment of oxidative stress parameters. **Results:** Pretreatment with sitagliptin remarkably alleviated the oxidative stress response induced by I/R in the jejunum manifested by a marked reduction of the pro-oxidant NOX-2 enzyme level, the lipid peroxidation marker, MDA content and the nitrosative stress indicator, NO content as well as replenishing the key cellular enzymatic antioxidant, SOD activity. **Conclusion:** Sitagliptin has a potential antioxidant effect against II/R injury in rats and thus we assume sitagliptin may be a beneficial prophylactic drug in patients at risk of intestinal I/R injury.

Keywords: *Sitagliptin; Ischemia; Reperfusion injury; Intestine; Oxidative stress*

INTRODUCTION

Ischemia/reperfusion (I/R) injury occurs when tissues or organs are subjected to a period of ischemia, followed by blood flow replenishment. Intestinal ischemia/reperfusion (II/R) injury is inevitable under numerous clinical conditions, such as in mesenteric artery embolism, intestinal transplantation, cardiopulmonary bypass, abdominal aortic aneurysm surgery, and traumatic or hemorrhagic shock[1]. Induction of ischemia and reperfusion to the small intestine, compared to other organs, is particularly ferocious as damage to the mucosal barrier integrity results in bacterial translocation, eventually leads to multiple organ failure, and death[2]. Delayed diagnosis in addition to the lack of effective treatment, the impact becomes detrimental with in-hospital mortality reaching 80%[3].

The molecular mechanisms underlying II/R injury have been extensively investigated over the past decades. It has been demonstrated that reactivation of aerobic metabolism to the small intestine by reperfusion after an adequate period of ischemia, is associated with a burst production of reactive oxygen and nitrogen species (ROS/RNS) such as superoxide anion, hydrogen

peroxide, hydroxyl radicals, and peroxynitrite to a level that overwhelms the endogenous antioxidant defense system such as superoxide dismutase (SOD) enzyme depletion, thus provoking further tissue damage beyond that induced by ischemia[4, 5].

A growing number of studies have reported that the nicotinamide adenine dinucleotide phosphate oxidase (NOX) enzyme, particularly highly expressed NOX-2 isoform is one of the major contributors of oxidative stress during II/R injury[6]. Furthermore, nitric oxide (NO) biosynthesis and its reaction are also thought to play an intriguing role in II/R injury mediating nitrosative stress. Therefore, targeting inhibition of ROS/RNS production at their sources have previously yielded favorable results in resisting II/R-induced tissue damage[7,8].

Sitagliptin is a drug that has been developed for the treatment of type-2 diabetes mellitus through enhancement of the endogenous incretin hormone, glucagon-like peptide-1 (GLP-1) via inhibiting the dipeptidyl peptidase-4 enzyme[9]. Interestingly, sitagliptin confers cytoprotective effects against I/R injury in different organs such as in the brain, heart, and the kidney[10-12]. Importantly, we recently showed the protective anti-inflammatory and anti-apoptotic effects of sitagliptin on II/R in rats that were coupled with potentiation of the GLP-1/GLP-1receptor axis[13]. Moreover, the improvement of oxidative stress status by sitagliptin, reported in previous studies, was settled with increasing GLP-1/GLP-1R expression[12,14]. Hence, these innovative findings prompt us to explore the possible antioxidant role of sitagliptin on II/R-mediated tissue injury**.**

MATERIAL AND METHODS

Animals

Adult male *Sprague-Dawley* rats (200-250 g) were obtained from the breeding unit of the Egyptian Organization of Biological Products and Vaccines (Helwan, Egypt). Rats were housed at the Faculty of Pharmacy, Helwan University, for one week before the experiment. Animals had free access to tap water and pelleted standard rat chow diet under controlled conditions (temperature of 22 ± 2 °C and 12 h light/dark cycles). Animal care and experimental protocols were approved by the ethics committee of scientific research, Faculty of Pharmacy, Helwan University (protocol number: 004A2018).

Induction of II/R injury

II/R injury was executed as previously described by[15]. Briefly, rats were anesthetized with thiopental (35 mg/kg, i.p) then a midline laparotomy of 4 cm length was made. Later, the superior mesenteric artery was determined and was clamped for 30min with an atraumatic microvascular clamp followed by a reperfusion period for 1 h by cautiously removing the clamp. The laparotomy was covered with warm saline-moistened gauze to prevent hypothermia. An electronic rectal thermometer was used for assessing systemic temperature. A heating lamp was used throughout the surgical operation to maintain body temperature at 37 ± 0.5°C.

Experimental design

Animals were divided into three experimental groups (n=15 in each group) as follows: (1) Sham group, the rats received 1% tween 80 (10 ml/kg; orally by gavage) for 2 weeks then underwent surgical procedures but without ischemic insult; (2) II/R group, the rats received 1% tween 80 (10 ml/kg; orally by gavage) for 2 weeks then subjected to 30 min intestinal ischemia and 1 h reperfusion; (3) Sitagliptin+ II/R group, the rats received sitagliptin (Sigma-Aldrich Chemical Company, USA; 300 mg/kg/day; orally by gavage) suspended in 1% tween 80 for 2 weeks before exposure to II/R injury. Sitagliptin dose was chosen based on previously published literature[10]. After the reperfusion period, animals were sacrificed by cervical dislocation. Small bowel samples from the jejunum were quickly harvested, washed in cold saline then preserved at -80°C for measurement of tissue parameters.

Measurement of biochemical parameters

For biochemical estimation, intestine tissues were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) to obtain 10% homogenates which were used for estimation of the following oxidative stress parameters:

Measurement of NOX-2

NOX-2 level was measured using a NOX-2 immunoassay kit (Biosource Enzo, USA; Cat.No. MBS2602532) according to the provided procedure.

Measurement of MDA

Enzyme immunoassay assay kit (Elbascience, USA; Cat.No. E-EL-0060) was used to determine MDA content according to the manufacturer's instructions.

Measurement of SOD

SOD activity was determined by the percent of inhibition of superoxide anion-dependent formation of water-soluble formazan dye from tetrazolium salt using (Abcam, USA; Cat.No. ab65354) colorimetric assay kit.

Measurement of NO

The intestine homogenate content of NO was measured using (Arbor colorimetric detection Kit, USA; Cat.No. K023-H1). In brief, NO is rapidly oxidized to nitrate and nitrite (totally referred to as NOx) in the presence of molecular oxygen. The assay determines

NOx content based on the reduction of any nitrate to nitrite utilizing nitrate reductase and NADH, followed by color development with Griess reagent (sulfanilamide and N-(1-naphthyl) ethylenediamine) in acidic medium to be measured spectrophotometrically at 540 nm.

Protein content

Protein content was evaluated according to the method of Lowry[16].

Statistical analysis

Data are expressed as mean ± SEM of 15 animals. Statistical comparisons between means were carried out using one-way analysis of variance (ANOVA), followed by Tukey- Kramer multiple comparison tests using Graph Pad Prism software (version 6). The statistical significance of difference was considered at P <0.05.

RESULTS

Effect of sitagliptin on the intestinal NOX-2 enzyme level in rats subjected to II/R injury

I/R induced a marked increase in intestinal NOX-2 enzyme level by 181% compared to the Sham group. Treatment with sitagliptin before II/R insult showed a significant decrease in the level of the NOX-2 enzyme by 26.8% compared to the II/R group as presented in **Figure 1.**

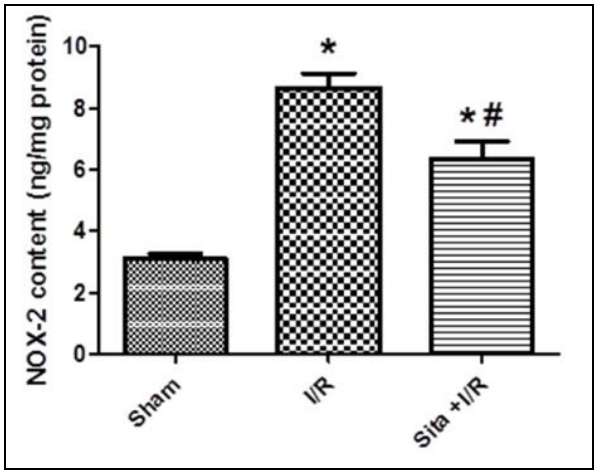

Figure 1. Effect of sitagliptin on intestinal NOX-2 content in rats subjected to II/R injury. Sita (300 mg/kg, orally by gavage) was administered for 2 weeks before induction of II/R. Data were expressed as mean ± SEM. Statistical comparisons between groups were carried out using ANOVA test followed by Tukey-Kramer multiple comparison tests. *significantly different with respect to the Sham group, # significantly different with respect to the II/R group (P <0.05). II/R= intestinal ischemia/ reperfusion; Sita=Sitagliptin

Effect of sitagliptin on intestinal MDA content in rats subjected to II/R injury

I/R produces a significant surge in intestinal MDA content by 2.3 fold as compared to the Sham group. Oral administration of sitagliptin before induction of II/R significantly decreased intestinal MDA content by 30.8% as compared to the II/R group as presented in **Figure 2**.

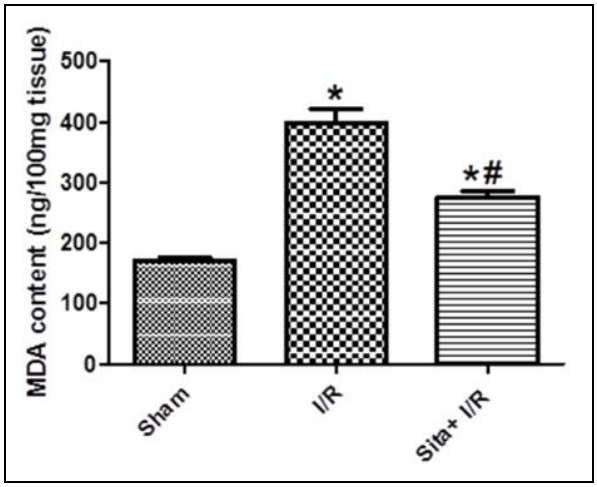

Figure 2. Effect of sitagliptin on intestinal MDA content in rats subjected to II/R injury. Sita (300 mg/kg, orally by gavage) was administered for 2 weeks before induction of II/R. Data were expressed as mean ± SEM. Statistical comparisons between groups were carried out using ANOVA test followed by Tukey-Kramer multiple comparison tests. *significantly different with respect to the Sham group, # significantly different with respect to the II/R group (P <0.05). II/R= ischemia/ reperfusion; Sita=sitagliptin

Effect of sitagliptin on intestinal SOD activity in rats subjected to II/R injury

I/R induced a remarkable decrease in intestinal SOD activity by 47.6% compared with the Sham group. However, pretreatment with sitagliptin could restore intestinal SOD activity to the level near to that of the Sham group as presented in **Figure 3**.

Effect of sitagliptin on intestinal NO content in rats subjected to II/R injury

Rats subjected to I/R showed a significant increase in intestinal NO content by 81.2% compared to the Sham group. On the other hand, pretreatment with sitagliptin significantly decreased intestinal NO content by 28.4% compared to the II/R group as presented in **Figure 4**.

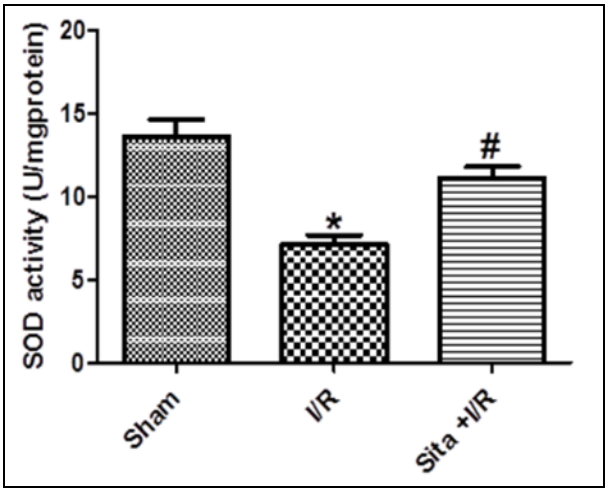

Figure 3. Effect of sitagliptin on intestinal SOD activity in rats subjected to II/R injury. Sita (300 mg/kg, orally by gavage) was administered for 2 weeks before induction of II/R. Data were expressed as mean ± SEM. Statistical comparisons between groups were carried out using ANOVA test followed by Tukey-Kramer multiple comparison tests. *significantly different with respect to the Sham group, # significantly different with respect to the II/R group (P <0.05). II/R= ischemia/ reperfusion; Sita=sitagliptin.

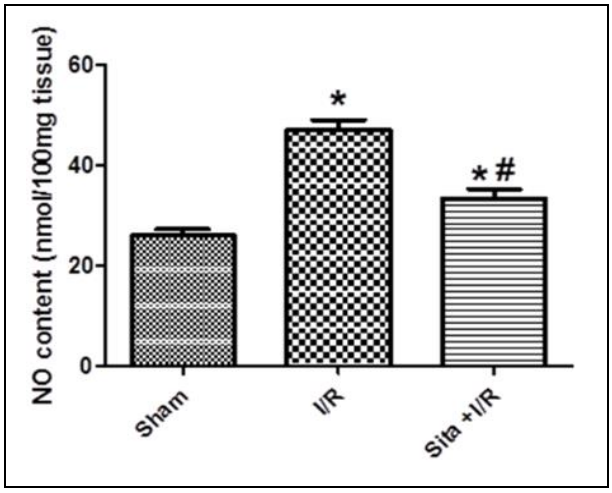

Figure 4. Effect of sitagliptin on intestinal NO content in rats subjected to II/R injury. Sita (300 mg/kg, orally by gavage) was administered for 2 weeks before induction of II/R. Data were expressed as mean ± SEM. Statistical comparisons between groups were carried out using ANOVA test followed by Tukey-Kramer multiple comparison tests. *significantly different with respect to the Sham group, # significantly different with respect to the II/R group (P <0.05). II/R= ischemia/ reperfusion; Sita=sitagliptin.

DISCUSSION

To the best of our knowledge, This current study is the first study that investigates the protective antioxidant impact of sitagliptin treatment on II/R injury in rats, which provides an extension to our recently published work that highlights sitagliptin's other pleiotropic effects such as anti-inflammatory and anti-apoptotic effects on the same induction model[13].

A huge body of experimental evidence has indicated that oxidative stress plays a quintessential role in the pathophysiology of intestinal I/R induced mucosal tissue damage[17,18]. Importantly, activated neutrophils reported being one of the major producers of reactive oxygen radicals during intestinal I/R concerning the activation of nicotinamide adenine dinucleotide phosphate oxidase-2 (NOX-2) enzyme[19,20]. The NOX-2 enzyme is a pro-oxidant enzyme that catalyzes the transfer of electrons from NADPH to O2, resulting in the generation of superoxide anion ($O2^{\bullet-}$)[21].

It is a multi-subunit enzyme complex composed of two membrane-bound catalytic subunits ($gp^{91\text{-}phox}$ and $p^{22\text{-}phox}$) and four cytosolic regulatory subunits $p^{47\text{-}phox}$, $p^{67\text{-}phox,}$ and $p^{40\text{-}phox}$, as well as the G-protein Rac^{22}. Several studies have revealed that induction of II/R caused a significant elevation of intestinal NOX-2 subunits $gp^{91\text{-}phox}$ and $p^{47\text{-}phox}$ proteins, drawing attention to its crucial role in intestinal I/R induced oxidative tissue damage[6,7,23]. In harmony, our current work displayed a significant upregulation of NOX-2 level in the intestine of rats subjected to I/R insult compared to that of the Sham group. On the contrary, sitagliptin pretreatment produces a substantial decrease in intestinal NOX-2 level compared with the II/R group. These results are in concordance with other studies[24,25]. Additionally, another supportive study conducted by Chang et al. reported that sitagliptin could suppress NOX-2 protein expression in the renal I/R model via the upregulation of GLP-1 and GLP-1 receptors[12]. Meanwhile, we recently showed that sitagliptin could successfully suppress the rigorous inflammatory response encountered by II/R through the upregulation of the transcription factor, nuclear factor kappa B (NF-κB) by activating the GLP-1/GLP-1R signaling pathway[13]. Interestingly, NF-κB is reported as one of the key regulators of the NOX-2 enzyme activity and NF-κB itself is activated by the NOX-2 enzyme through the produced ROS species[26]. Altogether, we can speculate that sitagliptin, through activation of GLP-1 signaling, could successfully inhibit the self-perpetuating activation cycle between NOX-2 and NF-κB, accordingly exerting its protective effects from mucosal oxidative tissue damage induced by II/R insult.

Superoxide anions are considered as the cornerstone of the damage associated with reperfusion of

ischemic intestine as it is the key driver for further oxygen-derived toxic species such as hydrogen peroxide, hydroxyl radical, hypochlorous acid and peroxynitrite. These toxic species cause extensive cell damage by binding to and altering the activities of cellular macromolecules through the processes of lipid peroxidation, protein and nucleic acid oxidation[27-29].

Lipid peroxidation (LP), for example, causes deterioration to the function of cell membranes by altering their physicochemical properties thus altering the flexibility, fluidity, and selective permeability of cell membranes ending in cell swelling, cytolysis, and finally cell death[30,31]. In the present work, MDA (major index of lipid peroxidation) was found to be markedly increased in rats subjected to II/R insult confirming the occurrence of extensive radical reactions that have a significant effect on LP, supporting the earlier findings[32,33].

Additionally, it has become clear that the excessive generation of free radicals will be accompanied by the depletion of antioxidant enzymes during the process of counteracting oxidative stress. SOD is considered one of the important enzymes in the supportive team of defense against reactive oxygen species where it catalyzes the dismutation of superoxide anions to hydrogen peroxide[31]. Data of the present investigation showed a distinct decrease in intestinal SOD activity in the II/R group compared to the Sham group, results that coincide with those reported in earlier studies[34,35]. Thus, the current work assures the overproduction of oxygen-derived free radicals and failure of enzymatic antioxidant defense mechanisms in the intestine following I/R induction, increasing gut susceptibility to oxidative cellular damage.

On the other hand, pretreatment with sitagliptin significantly attenuated MDA content, increases SOD activity recuperating its level to nearly that of the Sham group. The elevated SOD, when coupled to the reduced MDA content, implicates the strong antioxidant power of sitagliptin on II/R injury. In this way, our findings corroborated those of previous studies which showed the potential ability of sitagliptin to reduce the oxidative burden in several experimental models[36-38].

Intriguingly, many studies have pointed that II/R is also associated with excessive production of NO, revealing the important role of nitrosative stress in the pathogenic mechanism of II/R injury[39,40]. NO is generated by three isoforms of nitric oxide synthase (NOS): endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), with the latter as the most important isoform that is induced in response to II/R and responsible for the extensive NO release. This overproduced NO is found to be detrimental to intestinal integrity as it reacts with ROS, particularly with the superoxide anion forming peroxynitrite, an even more potent nitro-oxidative radical that is thought to react with all classes of biomolecules exacerbating mucosal tissue damage[41-43].

In support of these previous justifications, our results also showed that II/R-induced rats exhibited a marked increase in NO content compared to the Sham group. Conversely, groups pretreated with sitagliptin before II/R insult afforded a significant diminution of intestinal NO content compared to the I/R group. Bearing in mind that sitagliptin inhibited NF-κB activation in the same induction model[13], and in terms of the transcriptional control of iNOS induction, NF-κB has been heavily implicated, as multiple κB-binding sites are located in its promoter, thus down-regulation of NF-κB pathway by sitagliptin supposed to be a major factor in the suppression of II/R-induced nitrosative stress. This suggestion is strengthened by other findings and adds further confirmation to the antioxidant and anti-inflammatory effects of sitagliptin[38,44].

CONCLUSION

In summary, the findings of our study highlight the protective effects of sitagliptin against oxidative stress induced by II/R. This was evidenced in the reduction of the pro-oxidant NOX-2 enzyme level as well as replenishing the key cellular enzymatic antioxidant, SOD. These favorable effects coincided with a meaningful decline of the lipid peroxidation marker, MDA, and the nitrosative stress indicator, NO. Based on these findings, sitagliptin is worthy of further experimental and clinical studies before a conclusion can be reached on the utility of sitagliptin as preventive therapy for populations at risk of intestinal ischemia/reperfusion injury.

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Conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

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