

Dipeptidyl Peptidase-4 Inhibitor Sitagliptin Prevents Hepatic Injury via Liver Ischemia/reperfusion in Rats

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ABSTRACT

Background and Aim: Dipeptidyl peptidase-4 (DPP4, DPPIV, CD26, EC 3.4.14.5) was found out more than four decades ago as a serine protease that severs N-terminal dipeptides from peptide substrates. DPP-4 inhibitors have been used in many animal models of lung and heart illness, in which injury was obtained by an ischemic attack followed by following reperfusion. Here, we present the large body of experimental study that now gives irresistible evidence for the useful impact of DPP-4 targeting in IRI. In our study, we discuss the effect of DPP-4 inhibitor (Sitagliptin) on DPP-4 expression in rat model. **Methods:** We made a rat model of liver ischemia (90min)-reperfusion (180min), collected blood and liver samples after reperfusion. The possible inhibitory effect of Sitagliptin on DPP-4 in a rat model of hepatic IR damage was evaluated. Hepatic MDA levels were evaluated spectrophotometrically to know the degree of oxidizing reaction in liver. We evaluated the expression of tumor necrosis factor (TNF)- α and interleukin (IL)-6 in the model. We used HE staining to remark the change of liver morphologically. **Results:** Significantly, the expression of DPP-4 levels was declined after treatment with Sitagliptin in IR group. MDA, TNF- α and IL-6 levels were significantly increased in the IR group but decreased in the groups treated with Sitagliptin, 5mg/kg. HE staining show exact edema and necrosis were remarked in the IR group, but in the Sitagliptin pretreatment group, they were decreased. **Conclusion:** Our study showed that pretreatment with Sitagliptin might inhibit DPP-4 activation and reduce hepatic IR damage.

Key words: DPP-4, Hepatic IR injury, DPP-4 inhibitor, Sitagliptin.

INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) is a membrane-associated peptidase and this is known as CD26. DPP-4 is widely spread in organs throughout the body and presents pleiotropic effects by its peptidase activity.^[1-4] It is connected with immune stimulation, combining to and degradation of extracellular matrix, resistance to anti-cancer agents and lipid accumulation.^[5-8] In the liver, DPP-4 is presented to a high degree and recent accumulation shows that DPP-4 is connected with the development of various chronic liver diseases including hepatitis C virus infection, non-alcoholic fatty liver disease and hepatocellular carcinoma.^[9-12] In addition, DPP-4 is involved in hepatic stem cells and plays an important role in hepatic regeneration.^[8] Liver ischemia reperfusion (IR) injury is observed condition, which is caused by restoring blood supply after ischemia in liver which involved a series of pathophysiological processes, such as radical generation, neutrophil infiltration and release of inflammatory mediators. Liver surgery often needs clamping of the portal triad, reducing intraoperative blood loss and is necessary to cause liver IR injury which can increase postoperative liver insufficiency and even liver failure.^[13-14] However, there are no data connected oxidative injury and inflammation reaction with DPP-4 expression

and effect of DPP-4 inhibitor Sitagliptin in liver IR injury *in vivo*. In our study, we present the relation of oxidative injury and inflammation reaction with DPP4 expression and the effect of DPP-4 inhibitor (Sitagliptin) on DPP-4 expression in liver IR injury *in vivo* in rat model.²²⁷

MATERIALS AND METHODS

Animals

Male SD rat (200-250g) were obtained from the Laboratory Animal Center of Kim Il Sung University Pyongyang Medical College. Animals were fed a standard rodent diet and water and bred in a controlled environment with 12-hr light-dark cycles. They were treated as recommended in the Guide for the Care and Use of Laboratory Animals issued by the D.P.R.K Association of Laboratory Animal Care.

Liver IR Injury Model

We used an established rat model of hepatic IR injury, as described previously.^[13-14] Briefly, rat were anesthetized with isoflurane and injected with heparin (100 U/kg) and an atraumatic clip was used to interrupt the artery and portal venous blood supply to the left and middle liver lobes. After 90 mins of hepatic ischemia, the clamp was removed to generate hepatic

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reperfusion. Rats were sacrificed 180min after reperfusion for tissue and plasma collection. To evaluate the role of DPP-4 inhibitor, rat was pretreated with 5mg/kg of Sitagliptin at 20 minute prior to the ischemia insult. Sham rat underwent the same procedure but without vascular occlusion (n=10).

Liver malondialdehyde (MDA) levels

Hepatic MDA levels were evaluated spectrophotometrically to evaluate the degree of oxidizing reaction in liver.

Tumor Necrosis Factor: TNF and Interleukin-6(IL-6) in Liver

TNF- α and IL-6 levels were evaluated by enzyme-linked immunosorbent assay according to the manufacturer's protocol (Adlitteram Diagnostic Laboratories). One single treatment was performed on four individual.

Histology

Formalin-fixed, paraffin-embedded rat liver specimens were sectioned at 4 μ m and stained with HE. The sections were used for histopathologic examinations by light microscopy.

Statistical Analysis

Statistical analysis was carried out using SPSS software, version 14.0(SPSS Inc. Chicago, Ill, USA). Results are expressed as means standard deviations. Parameters were analyzed by Student *t* test. For the above parameters, $P < 0.05$ was considered to be statistically significant.

RESULTS

MDA, TNF- α and IL-6 Levels in Liver

The levels of MDA were significantly increased in the IR group (control group), but significantly decreased in groups pretreated with 5mg/kg Sitagliptin (Figure 1). The TNF- α and IL-6 levels in the IR group were significantly increased, but significantly decreased in groups pretreated with 5mg/kg Sitagliptin (Figure 2).

Histological Changes

Apparent edema and necrosis were observed in the IR group (Figure 3 B). In the Sitagliptin pretreatment group, edema and necrosis in IR modes were reduced. Disrupted lobular architecture and apparent edema were observed in the Sitagliptin group (Figure 3 C).

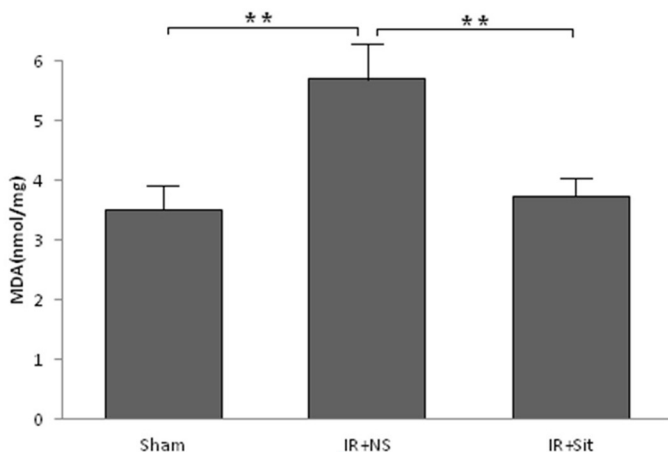


Figure 1: The levels of malondialdehyde (MDA) in sham group, ischemia-reperfusion (IR) group and 5mg/kg concentration of Sitagliptin pretreatment groups.

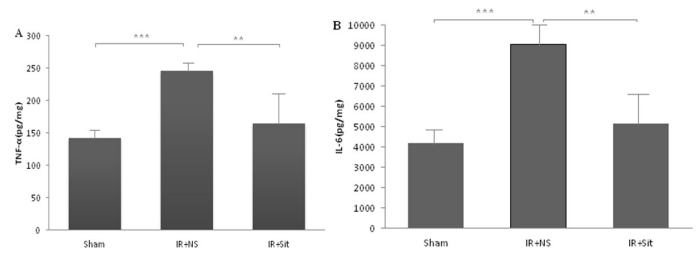


Figure 2: Serum tumor necrosis factor (TNF)- α and interleukin (IL)-6 in the sham group, ischemia-reperfusion (IR) group (control group) and groups pretreated with 5mg/kg concentrations of Sitagliptin.

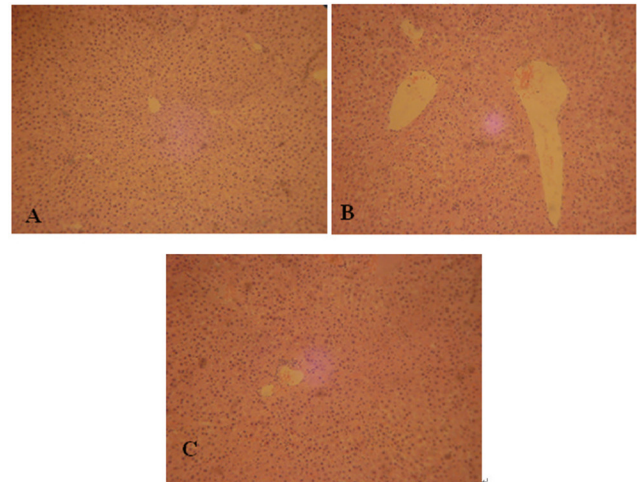


Figure 3: Representative hematoxylin and eosin staining in the sham group (A), ischemia-reperfusion (IR) group (B) and Sitagliptin pretreatment group (C) at 180min after reperfusion.

DISCUSSION

Recently, researchers use partial hepatic ischemia models of rats rather than total hepatic ischemia models and this is because the total ischemia models in liver frequently have hypotension, systemic vascular congestion and also high mortality.^[16] Therefore, in our study, we choose a partial ischemia model to derive hepatic IR injury.

It is clear that Dipeptidyl peptidase-4 (DPP-4/DPPIV/CD26) cleaves off N-terminal dipeptides from peptides with preferably proline or alanine at the penultimate position.^[17] Many DPP-4 inhibitors like sitagliptin, vildagliptin, saxagliptin and linagliptin are available for the treatment of type 2 diabetes. Their pharmacological action is based on the reduced cleavage of incretin hormone glucagon-like peptide-1 (GLP-1) by DPP-4, preserving the insulinotropic action of this peptide.^[18] Recently, many studies have done regarding DPP-4 inhibitors for their applicability in other conditions pathologically, both in animal studies and in clinical settings.^[19]

IRI is characterized by an initial restriction of blood supply to an organ and it is followed by the subsequent reperfusion with concomitant reoxygenation. During ischemia, tissue hypoxia is caused by the severe imbalance of metabolic supply and demand. Restoration of the blood flow and reoxygenation is often accompanied by an exacerbation of tissue damage and a profound inflammatory response.^[20] IRI is connected with modified local cytokine/chemokine secretion patterns, increased neutrophil recruitment, free-radical accumulation, lipid peroxidation and impairment of functional and structural integrity of the organ.^[20] Our study

showed that the content of MDA, TNF- α and IL-6 in the liver tissue are increased in hepatic ischemia/reperfusion injury model than in the normal one and they were decreased by the injection of Sitagliptin, one of the DPP4 inhibitor. The relevance of DPP4 as a target in IRI has been presented in several animal studies, mostly myocardial infarction.^[21-31] and experimental lung Tx,^[32-36] either using DPP4 inhibitor treatment or DPP4 knock out animals. Apart from these animal studies, in patients with coronary artery disease,^[37] one study in humans showed cardio-protection by sitagliptin. Another research reported a reduction of the infarct size after myocardial IRI upon DPP4 inhibitor treatment.^[29] The renal IRI studies were either performed in diabetic,^[38] or non-diabetic animals,^[39] both showing a reduction in serum creatinine levels upon DPP4 inhibition. Sauvé et al. discovered a decrease of mortality both in DPP-4 and sitagliptin-treated mice.^[23] DPP4 inhibitors have capable ability to protect the heart, kidney and lungs against IRI in preclinical models.

There are a few data that is related to DPP-4 in the liver model of ischemia/reperfusion injury. The DPP4 expression is increased in the model of liver ischemia/reperfusion injury, resulting in the increase of oxidative procedure and inflammation morphologic change in the liver tissue. These changes were clearly reduced by Sitagliptin which is known to be one of DPP-4 inhibitor. These demonstrated that Sitagliptin reduced the content of MDA, TNF- α and IL-6 and also improved the pathophysiologic findings in liver tissue, inhibiting the expression of DPP4.

In our study, we presented that pretreatment with DPP4 inhibitor Sitagliptin results in reduced MDA, TNF- α and IL-6 production in hepatic IR injury *in vivo* and this is consistent with previous studies.^[40] In addition, we also demonstrated that pretreatment with Sitagliptin results in significantly reduced proinflammatory cytokine production in hepatic IR injury models *in vivo* and this is supporting that Sitagliptin might promote anti-inflammatory by inhibiting DPP-4 *in vivo*.

Our data clearly show that Sitagliptin may inhibit expression of DPP-4 in hepatic IR. In addition, we conclude that targeting DPP-4 represents as a useful approach to promoting hepatic IR injury. These results give the rationale for promoted approaches to decline hepatic IR injury.

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Non.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

DPP-4: Dipeptidyl peptidase-4.

SUMMARY

Dipeptidyl peptidase-4 (DPP4, DPPIV, CD26, EC 3.4.14.5) was found out more than four decades ago as a serine protease that severs N-terminal dipeptides from peptide substrates. DPP-4 inhibitors have been used in many animal models of lung and heart illness, in which injury was obtained by an ischemic attack followed by following reperfusion. Here, we present the large body of experimental study that now gives irresistible evidence for the useful impact of DPP-4 targeting in IRI. In our study, we discuss the effect of DPP-4 inhibitor (Sitagliptin) on DPP-4 expression in rat model. We made a rat model of liver ischemia (90min)-reperfusion (180min), collected blood and liver samples after reperfusion. The possible inhibitory effect of Sitagliptin on DPP-4 in a rat model of hepatic IR damage was evaluated. Hepatic MDA levels were evaluated spectrophotometrically to know the degree of oxidizing reaction in liver. We evaluated the expression of tumor necrosis factor (TNF)- α and inter-

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