The Effect of Sitagliptin on Hepatic Ischemic Reperfusion Injury in Rats

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Background: Dipeptidyl peptidase-4 (DPP-4, 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 the 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 ischemia/reperfusion injury. In this study, we discuss the effect of DPP-4 inhibitor (Sitagliptin) on DPP-4 expression in the rat model. Materials and Methods: We made a rat model of liver ischemia (90 min)-reperfusion (180 min), collected blood and liver samples after reperfusion. The possible inhibitory effect of Sitagliptin on DPP-4 in a rat model of hepatic ischemia-reperfusion (IR) damage was evaluated. Hepatic malondialdehyde (MDA) levels were evaluated spectrophotometrically to know the degree of oxidizing reaction in the liver. We evaluated the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the model. We used hematoxylin and eosin (H and E) staining to remark the change of liver morphologically. Results: Significantly, the expression of DPP-4 levels was declined after treatment with Sitagliptin in the IR group. MDA, TNF-α, and IL-6 levels were significantly increased in the IR group but decreased in the groups treated with Sitagliptin, 5 mg/kg. H and E staining show exact edema and necrosis were remarked in the IR group, but in the Sitagliptin pretreatment group, they were decreased. Conclusion: The study showed that pretreatment with Sitagliptin might inhibit DPP-4 activation and reduce hepatic IR damage.

Keywords: Dipeptidyl peptidase-4, dipeptidyl peptidase-4 inhibitor, hepatic ischemia-reperfusion injury, 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 the 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, nonalcoholic fatty liver disease,[9,10] and hepatocellular carcinoma.[11,12] In addition, DPP-4 is involved in hepatic stem cells and plays an important role in hepatic regeneration.[8]

Liver ischemia-reperfusion injury (IRI) is observed condition, which is caused by restoring blood supply after ischemia in the 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 selected organs to allow for liver resection, and this is often followed by reperfusion. This ischemia/reperfusion injury is characterized by the release of inflammatory mediators, which leads to damage of liver parenchyma.[13,14] It is important to develop effective therapeutic strategies to prevent and treat liver injury after ischemia/reperfusion.

Materials and Methods: We made a rat model of liver ischemia (90 min)-reperfusion (180 min), collected blood and liver samples after reperfusion. The possible inhibitory effect of Sitagliptin on DPP-4 in a rat model of hepatic ischemia-reperfusion (IR) damage was evaluated. Hepatic malondialdehyde (MDA) levels were evaluated spectrophotometrically to know the degree of oxidizing reaction in the liver. We evaluated the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the model. We used hematoxylin and eosin (H and E) staining to remark the change of liver morphologically. Results: Significantly, the expression of DPP-4 levels was declined after treatment with Sitagliptin in the IR group. MDA, TNF-α, and IL-6 levels were significantly increased in the IR group but decreased in the groups treated with Sitagliptin, 5 mg/kg. H and E staining show exact edema and necrosis were remarked in the IR group, but in the Sitagliptin pretreatment group, they were decreased. Conclusion: The study showed that pretreatment with Sitagliptin might inhibit DPP-4 activation and reduce hepatic IR damage.

Keywords: Dipeptidyl peptidase-4, dipeptidyl peptidase-4 inhibitor, hepatic ischemia-reperfusion injury, sitagliptin
portal triad, reducing intraoperative blood loss, and is necessary to cause liver IRI which can increase postoperative liver insufficiency and even liver failure.\textsuperscript{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 IRI \textit{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 IRI \textit{in vivo} in rat model 227.

**Materials and Methods**

**Animals**

Male SD rats (200–250 g) 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 h light–dark cycles. All animal procedures were approved by the Institutional Animal Care Committee and conducted in accordance with the Kim Il Sung University Pyongyang Medical College Guidelines for the Care and Use of Laboratory Animals.

**Liver ischemia-reperfusion injury model**

We used an established rat model of hepatic IRI, as described previously.\textsuperscript{13,14} Briefly, rats 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 min of hepatic ischemia, the clamp was removed to generate hepatic reperfusion. Rats were sacrificed 180 min after reperfusion for tissue and plasma collection. To evaluate the role of DPP-4 inhibitor, rats were pretreated with 5 mg/kg of Sitagliptin at 20 min before the ischemia insult. Sham rat underwent the same procedure but without vascular occlusion \((n = 10)\).

**Serum levels of alanine aminotransferase and aspartate aminotransferase**

The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are one index of hepatocyte injury. A standard automatic analyzer (Hitachi 7600–10, Hitachi High-Technologies Corporation, Japan) was used to determine the serum levels of ALT and AST.

**Liver malondialdehyde levels**

Hepatic malondialdehyde (MDA) levels were evaluated spectrophotometrically to evaluate the degree of oxidizing reaction in the liver as previously described.\textsuperscript{15} The absorbance of the upper layer was read at 532 nm with a spectrophotometer (Kadas 100, Dr. Lange, AG Zurich, Zurich, Switzerland), and the results expressed as nanomoles of MDA per liter of wet liver tissue.

**Tumor necrosis factor-α and interleukin-6 in the liver**

Tumor necrosis factor-α (TNF-α) concentration in serum and mesenteric lymph was determined by using rat TNF-enzyme-linked immunoabsorbent assay kit (LIFEKEY Biotech, Co., USA) according to the manufacturer’s protocol. Interleukin-6 (IL-6) levels were evaluated by IL-enzyme-linked immunosorbent assay according to the manufacturer’s protocol (Adlitteram Diagnostic Laboratories). One single treatment was performed on four individual wells.

**Histology**

Formalin-fixed, paraffin-embedded rat liver specimens were sectioned at 4 μm and stained with hematoxylin and eosin. Liver sections from the left lobe were stained with hematoxylin (Muto Pure Chemicals, Tokyo, Japan) and eosin (Wako, Osaka, Japan). The sections were used for histopathologic examinations by light microscopy \((\times 100)\).

**Statistical analysis**

Statistical analysis was performed using the SPSS software, version 14.0 (SPSS Inc., Chicago, Ill., USA). Results are expressed as means and standard deviations. Parameters were analyzed using Student’s \(t\)-test. For the above parameters, \(P < 0.05\) was considered to be statistically significant.

**Results**

**Serum alanine aminotransferase and aspartate aminotransferase levels**

The levels of ALT and AST were significantly increased in the IR group (control group) but significantly decreased in groups pretreated with 5 mg/kg Sitagliptin [Figure 1].

**Malondialdehyde, tumor necrosis factor-α, and interleukin-6 levels in the liver**

The levels of MDA were significantly increased in the IR group (control group) but significantly decreased in groups pretreated with 5 mg/kg Sitagliptin [Figure 2]. The TNF-α and IL-6 levels in the IR group were significantly increased but significantly decreased in groups pretreated with 5 mg/kg Sitagliptin [Figure 3].

**Histological changes**

Apparent edema and necrosis were observed in the IR group [Figure 4b] compared to sham group [Figure 4a]. 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 4c].
**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. Therefore, in this study, we choose a partial ischemia model to derive hepatic IRI.

It is clear that DPP-4/DPPIV/CD26 cleaves off N-terminal dipeptides from peptides with preferably proline or alanine at the penultimate position. Many DPP-4 inhibitors such as 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 by DPP-4, preserving the insulinotropic action of this peptide. Recently, many studies have done regarding DPP-4 inhibitors for their applicability in other conditions pathologically, both in animal studies and in clinical settings.

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 profound inflammatory response. 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.

The study showed that the content of MDA, TNF-α, and IL-6 in the liver tissue are increased in hepatic IRI model than in the normal one and they were decreased by the injection of Sitagliptin, one of the DPP4 inhibitors. The relevance of DPP4 as a target in IRI has been presented in several animal studies, mostly myocardial infarction and experimental lung Tx, either using DPP4 inhibitor treatment or DPP4 knock out animals. Apart from these animal studies, in patients with coronary artery disease, one study in humans showed cardioprotection by sitagliptin. Another research reported a reduction of the infarct size after myocardial IRI on DPP4 inhibitor treatment. The renal IRI studies were either performed in diabetic or nondiabetic animals, both showing a reduction in serum creatinine levels on DPP4 inhibition. Sauvé *et al.*

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**Figure 1:** The serum level of alanine aminotransferase and alanine asparaginic acid (aspartate aminotransferase) in the sham group, ischemia-reperfusion group (control group), and groups pretreated with 5 mg/kg concentrations of Sitagliptin. The alanine aminotransferase and aspartate aminotransferase levels in the ischemia-reperfusion group were significantly increased, but significantly decreased in groups pretreated with 5 mg/kg Sitagliptin. *P < 0.05

**Figure 2:** The levels of malondialdehyde in sham group, ischemia-reperfusion group, and 5 mg/kg concentration of Sitagliptin pretreatment groups. **P < 0.01

**Figure 3:** Serum tumor necrosis factor-α (a) and interleukin-6 (b) in the sham group, ischemia-reperfusion group (control group), and groups pretreated with 5 mg/kg concentrations of Sitagliptin. **P < 0.01, ***P < 0.001
discovered a decrease of mortality both in DPP-4 and sitagliptin-treated mice. 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 IRI. The DPP4 expression is increased in the model of liver IRI, resulting in an 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 the DPP-4 inhibitors. These demonstrated that Sitagliptin reduced the content of MDA, TNF-α, and IL-6 and also improved the pathophysiology findings in liver tissue, inhibiting the expression of DPP4.

In this study, we presented that pretreatment with DPP4 inhibitor Sitagliptin results in reduced MDA, TNF-α, and IL-6 production in hepatic IRI in vivo and this is consistent with previous studies. In addition, we also demonstrated that pretreatment with Sitagliptin results in significantly reduced proinflammatory cytokine production in hepatic IRI 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 a useful approach to promoting hepatic IRI. These results give the rationale for promoted approaches to decline hepatic IRI.

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**Conflicts of interest**

There are no conflicts of interest.
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