# ORIGINAL ARTICLES

## Effects of addition of a dipeptidyl peptidase IV inhibitor to metformin on sirolimus-induced diabetes mellitus

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**The guideline for the management of new-onset diabetes after transplantation recommends metformin (MET) as a first-line drug, and addition of a second-line drug is needed to better control of hyperglycemia. We tested the effect of addition of a dipeptidyl peptidase IV (DPP IV) inhibitor to MET on sirolimus (SRL)-induced diabetes mellitus (DM). In animal model of SRL-induced DM, MET treatment improved pancreatic islet function (blood glucose level and insulin secretion) and attenuated oxidative stress and apoptotic cell death. Addition of a DPP IV inhibitor to MET improved these parameters more than MET alone. An in vitro study showed that SRL treatment increased pancreas beta cell death and production of reactive oxygen species (ROS), and pretreatment of ROS inhibitor, or p38MAPK inhibitor effectively decreased SRL-induced islet cell death. Exendin-4 (EXD), a substrate of DPP IV or MET significantly improved cell viability and decreased ROS production compared with SRL treatment, and combined treatment with the 2 drugs improved both parameters. At the subcellular level, impaired mitochondrial respiration by SRL were partially improved by MET or EXD and much improved further after addition of EXD to MET. Our data suggest that addition of a DPP IV inhibitor to MET decreases SRL-induced oxidative stress and improves mitochondrial respiration. This finding provides a rationale for the combined use of a DPP IV inhibitor and MET in treating SRL-induced DM. (Translational Research 2016;174:122–139)**

**Abbreviations:** CNI = calcineurin inhibitors; NODAT = new-onset diabetes after transplantation; PTDM = posttransplant diabetes mellitus; SRL = sirolimus; DPP IV = dipeptidyl peptidase IV; DM = diabetes mellitus; LC = LC15-0444; MET = metformin; IPGTT = intraperitoneal glucose tolerance test; ITT = insulin tolerance test; GSIS = glucose-stimulated insulin secretion; EXD = exendin-4; ROS = reactive oxygen species; 8-OHDG = 8-hydroxy-2′-deoxyguanosine

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## AT A GLANCE COMMENTARY

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### Background

The protective role of dipeptidyl peptidase IV (DPP IV) inhibitors against diverse types of injury is being increasingly recognized. This study evaluated whether a DPP IV inhibitor can protect against sirolimus (SRL)-induced pancreatic islet injury.

### Translational Significance

We demonstrate that addition of a DPP IV inhibitor to metformin improved glucose control by decreasing oxidative stress in an animal model of SRL-induced diabetes mellitus. At the subcellular level, impaired mitochondrial respiration caused by SRL improved markedly after combined treatment with a DPP IV inhibitor and metformin. This finding may provide a rationale for the clinical use of a DPP IV inhibitor in the treatment of SRL-induced diabetes mellitus.

## INTRODUCTION

New-onset diabetes after transplantation (NODAT) is a serious complication that can adversely affect the survival of the transplant recipient and graft after solid organ transplantation.[1] The causes of NODAT are multifactorial,[2] but the use of an immunosuppressive regimens is a major contribute to the risk factors for NODAT. Among these drugs, high-dose steroids and calcineurin inhibitors are well-known causes of NODAT.[3-5] Sirolimus (SRL) was initially regarded as a nondiabetogenic immunosuppressant, but a clinical study has shown that switching from a calcineurin inhibitor to SRL can cause or further aggravate NODAT, and experimental study has shown that SRL itself causes diabetes mellitus (DM) by impairing insulin secretion or by directly injuring pancreatic islet beta cells.[6,7]

The guideline for NODAT management is based on type 2 DM.[8] In the guideline, metformin (MET) is recommended as a first-line drug for type 2 DM, and addition of a second-line drug is suggested to achieve better control of hyperglycemia. Among these second-line drugs, dipeptidyl peptidase IV (DPP IV) inhibitors have recently gained considerable interest for the treatment of type 2 DM and NODAT.[9] In addition to providing excellent glucose control, DPP IV inhibitors may have pleiotropic effects, such as anti-inflammatory, antiapoptotic, and immunomodulatory actions. These protective effects of DPP IV inhibitors have been studied in models of various renal injuries,[10-12] DM,[13,14] hepatic impairment,[15] and cardiovascular disease.[16,17] Using a well-known animal model, we recently demonstrated that DPP IV inhibitors protect against tacrolimus-induced pancreatic islet and renal injury through their antiapoptotic and antioxidative actions.[18,19]

Considering these findings, we tested whether addition of DPP IV inhibitors to MET would protect against SRL-induced pancreatic islet injury. First, we evaluated whether a DPP IV inhibitor would have a protective effect in an experimental model of SRL-induced DM. Second, we observed whether a DPP IV inhibitor would have a direct protective effect on pancreatic islet cell viability and production of reactive oxygen species (ROS). Third, we evaluated the effects of a DPP IV inhibitor on mitochondrial function by measuring mitochondrial respiration. The results of our study demonstrate that DPP IV inhibitors protect against SRL-induced pancreatic islet cell injury and provide a rationale for the addition of a DPP IV inhibitor to MET in the treatment of SRL-induced DM in clinical practice.

## METHODS

**Animal care and drug use.** The experiment protocol (CUMC-2014-0047-03) was approved by the Animal Care and Use Committee of the Catholic University of Korea, and all procedures performed in this study were in accordance with ethical guidelines for animal studies. Eight-week-old male Sprague Dawley rats (Charles River Technology, Seoul, Korea) that initially weighed 220–230 g were housed in cages (Nalge Co., Rochester, NY) in a controlled temperature and light environment at the Catholic University of Korea's animal care facility. The rats received a low-salt diet (0.05% sodium, Teklad Premier, Madison, Wis). SRL (Wyeth-Ayerst Research, Princeton, NJ) was diluted in Tween 80 (10%), N, N-dimethylacetamide (20%), and polyethylene glycol 400 (70%) to a final concentration of 0.3 mg/kg. The DPP IV inhibitor LC15-0444 (LC) was kindly supplied by LG Life Sciences (Seoul, Korea) and was diluted in drinking water to a final concentration of 5 mg/kg. MET (Dongwha Pharmaceutical Co., Seoul, Korea) was diluted in drinking water to a final concentration of 250 mg/kg.

**Experimental design.** The study was designed to determine whether the combination of LC and MET would result in less glycemia than would LC or MET administered alone in rats with SRL-induced DM. A total of 45 male Sprague Dawley rats were randomized to 5 groups each containing 9 rats. The rats were treated with SRL