Discussion & Conclusion
7. Discussion

DPP-4 inhibitors augment the effects of incretin hormones by prolonging their half-life and represent a new therapeutic approach for the treatment of type 2 diabetes (Pratley and Salsali., 2007). Drawback of vildagliptin includes short half life; inefficiently inhibit gastrointestinal functions, and diabetic complications (He et al., 2009; Adrian et al., 2007). Therefore, the vildagliptin analogue PKF-275-055 was synthesized as a selective, long-acting inhibitor of dipeptidyl peptidase-4 for the treatment of diabetes and its complications (Bianchi et al., 2011). In the current study, vildagliptin analogue PKF-275-055 was tested after chronic dosing (once a day) in preclinical models of streptozotocin induced diabetes mellitus. Neonatal–STZ wistar model is well characterized model of diabetes mellitus. Neonatal–STZ rats develop persistent diabetes rapidly after 6 weeks of age, and showed diabetes like symptoms such as lack of insulin release in response to glucose, glucose intolerance, raised glycosylated hemoglobin, and depletion of pancreatic insulin store (Weir et al., 1981; Daniel., 1991; Masiello et al., 1998). The present study demonstrated that the DPP-IV inhibitors PKF-275-055 have a glucose tolerance–improving effect comparable with or superior to that of vildagliptin, which suggests their usefulness as a therapeutic agent for diabetes mellitus.

In the present study, we investigated the antihyperglycemic effects of PKF-275-055 in streptozotocin induced diabetic rats, which exhibited a mild decline in glucose tolerance due to loss of early-phase insulin secretion (Takasaki et al., 2004). These diabetic rats experienced an approximately 70 % decrease in pancreatic insulin content. Furthermore, fasting plasma GLP-1 levels before glucose loading did not differ between normal and diabetic rats. PKF-275-055 caused significant decreases in the blood glucose levels during both the 1st and 2nd OGTT in treated diabetic rats. In contrast, vildagliptin had no significant effect during the 2nd OGTT in diabetic rats. Furthermore, fasting plasma DPP-IV levels did not differ between normal and diabetic rats. At the dose of 10 mg/kg, both Vildagliptin and PKF-275-055 significantly inhibited (> 50 % inhibition) plasma DPP-IV activity during both the 1st and 2nd OGTT in diabetic rats. In present study, we observed that PKF-275-055 is a selective DPP-IV inhibitor and exhibits a potent and long-acting antihyperglycemic effect based on a glucose-dependent insulinotropic action associated with increases in plasma GLP-1 levels during both the 1st and 2nd OGTT in diabetic rats.
than vildagliptin. From the chronic study, it was clear that PKF-275-055 rapidly inhibited plasma DPP-4 activity in diabetic rats in a dose-related manner. This action of PKF-275-055 was accompanied by 1) a marked increase in the glucose-stimulated levels of intact GLP-1, 2) enhanced glucose-stimulated insulin levels, and 3) a marked decrease in glucose excursions after an oral glucose challenge. The minimum effective dose of PKF-275-055 to inhibit DPP-4, to augment intact GLP-1, to improve β-cell function, and to reduce glucose excursions was 1 mg/kg, and a dose of 10 mg/kg exerted maximal effects on all parameters. These findings are consistent with those of several earlier studies using other DPP-4 inhibitors in glucose intolerant rodents, including Zucker fatty rats (Takasaki et al., 2004; Balkan et al., 1999), high-fat-fed rats (Mitani et al., 2002) and mice (Ahre´n et al., 2000), streptozotocin-nicotinamide–induced mildly diabetic mice (Akiko et al., 2008) and aged rats (Mitani et al., 2002).

In present study, Body weight gain was observed in rats treated for 8 weeks with PKF-275-055 (10 mg/kg) averaged 239.2±5.54g (~20%) vildagliptin (10 mg/kg) averaged 234.2±4.36 g (~17%). This was significantly different from weight gain in the vehicle-treated diabetic rats, which averaged 200.8±5.23g.

In normal rats, PKF-275-055 and vildagliptin significantly inhibited increases in the blood glucose level during the OGTT. In contrast, both DPP-IV inhibitors had no significant effect on fasting blood glucose levels (unpublished data). Sulfonylureas, strongly inhibit ATP-sensitive K+ channel activity by binding to the high-affinity sulfonylurea receptors in pancreatic β-cells, which stimulates insulin secretion glucose-independently. Hypoglycemia has been reported as a side effect with the use of sulfonylureas in diabetic patients (Stahl and Berger., 1999). But, DPP-IV inhibitors has no effect on fasting blood glucose levels, there should be no risk of hypoglycemia, which, unlike the existing insulin secretagogue sulfonylurea, makes it safe for use as an antihyperglycemic.

Another incretin, GIP, is secreted from K cells in the duodenum and jejunum in response to oral ingestion of nutrients (Yip and Wolfe., 2000). Like GLP-1, GIP potentiates glucose-stimulated insulin release, and is degraded by DPP-IV to a biologically inactive form [GIP (3–42)] (Kieffer et al., 1995). It has been reported that DPP-IV inhibitors increased the plasma insulin level and decreased the postprandial blood glucose level in
both GLP-1 receptor deficient mice and GIP receptor-deficient mice (Hansotia et al., 2004). However, in double GLP-1 and GIP receptor-deficient mice, the DPP-IV inhibitors had no postprandial blood glucose-lowering effect. These results suggest that both GLP-1 and GIP contribute to the improvement in glucose tolerance elicited by DPP-IV inhibitors. Although the effects of vildagliptin and PKF-275-055 on plasma GIP levels were not investigated in this study, GIP may contribute to the antihyperglycemic efficacy of PKF-275-055 in diabetic rats.

In addition to potentiating the effects of GLP-1 and GIP, DPP-IV inhibitors may also prolong the actions of other peptide hormones, such as neuropeptide Y, substance P and growth hormone-releasing hormone, as well as chemokines (Drucker., 2003). Therefore, potential side effects associated with the reduced degradation of other peptide hormones and chemokines need to be considered. However, animals lacking DPP-IV consistently display healthy phenotypes (Marguet et al., 2000; Nagakura et al., 2001), and to date, no serious side effects due to DPP-IV inhibition have been reported in clinical studies (Kim et al., 2005; Ahren et al., 2004). Hence, DPP-IV inhibition may not produce undesirable changes in downstream biological pathways, despite altering the relative levels of intact- to-cleaved peptide substrates. In addition, PKF-275-055 showed significant inhibitory activity for DPP-IV than vildagliptin, but not showed any undesirable effects in 8 weeks chronic treatment.

GLP-1 not only stimulates insulin secretion glucose dependently, but also acts as a physiological mediator for various gastrointestinal functions. Recent studies revealed that, in addition to the incretin effect, exogenous GLP-1 or GLP-1 derivatives also caused a delay in gastric emptying and intestinal transit rates, which was considered to be partially responsible for the inhibition of postprandial hyperglycemia (Nauck et al., 1997; Delgado et al., 2002). From the present study, we observed delay in gastric emptying when the incretin effect was induced through increased endogenous GLP-1 levels after administration of a DPP-IV inhibitor (Balkan et al., 1999). In this study, PKF-275-055 dose-dependently inhibited gastric emptying and small intestinal transit rates, with significance at doses of 1mg/ kg or higher. In contrast, vildagliptin also showed dose-dependent inhibition of gastric emptying, but values were not statistically significant; also, vildagliptin did not significantly influence small intestinal transit rates. In meta-
analysis of randomize clinical trials, Monami *et. al* reported nausea, headache, and gastrointestinal disturbances resulting from a DPP-IV inhibitor (Monami et al., 2010). This may be due to a delay in gastric emptying and reduced small intestinal transit, which leads to a feeling of fullness.

Vildagliptin has been shown to lower blood glucose and HbA1c in human studies (Ahren et al., 2004; Ahren et al., 2004a) and decrease plasma glucose and increase plasma insulin in rodents (Ahren et al., 2005; Burkey et al., 2005; Flock et al., 2007). In current study, both vildagliptin and PKF-275-055 at the dose of 3 and 10 mg/kg significantly inhibited increase in HbA1c level and HOMA-Index compared with diabetic group. Furthermore, dose dependent improvement in β-cell function showed by both the drug but values were not statistically significant as compared with diabetic group. Vildagliptin, as well as other DPP-IV inhibitors, has been shown to increase proliferation, β-cell mass (BCM), and pancreatic insulin content and decrease apoptosis (Mentlein., 1999; Ahren et al., 2007; Cheng et al., 2008; Moritoh et al., 2008; Mu et al., 2006; Pospisilik et al., 2003). In the present study, Eight week treatment with PKF-275-055 showed effects on proliferation, BCM, and pancreatic insulin content.

As expected, islets of normal rats comprised a large insulin positive β-cell core surrounded by a mantle of glucagon positive α-cells. In contrast, islets from vehicle-treated diabetic rats contained many more glucagon positive α-cells, which infiltrated the islet including the central core. The 8-week treatment with the doses 10 mg/kg of vildagliptin and PKF-275-055 did not affect the number of α-cells in the islet core, but stimulated β-cells regeneration. Vildagliptin and PKF-275-055 at the dose of 10 mg/kg efficiently reduces pancreatic cell apoptosis in diabetic treated rats.

Studies in rodent islets have shown that GLP-1 acts to directly regulate the insulin gene and upregulates genes involved in insulin biosynthesis and downregulate genes involved in insulin resistance (Drucker et al., 1987; Wang et al., 1999; Wang et al., 1997). Therefore, we measured GLUT-4 mRNA, liver SREBP1C mRNA, pancreatic GLP-1 mRNA concentrations; and muscle GLUT-4 and liver GLUT-2 expression.

GLP-1 has rapid and potent effects to regulate insulin secretion in vivo and in vitro. Not only does GLP-1 stimulate glucose-dependent insulin secretion, it also increases insulin biosynthesis, decreases the proinsulin/insulin ratio, stimulates proliferation and decreases
apoptosis, and increases glucokinase and the glucose transporter GLUT4 mRNA levels (Holst, 2007). From the present study, we observed that vildagliptin treated diabetic rats showed 10.68 folds increase GLP-1R mRNA concentration and PKF-275-055 treated diabetic rats showed 4 times increase GLP-1R mRNA concentration in the pancreatic β cells than diabetic animals.

In diabetic rats, both vildagliptin and PKF-275-055 treatment seems to act in liver GLUT2 and muscle GLUT4 expression at the post-transcriptional level, by stimulating the translational process toward normalization of glucotransporter protein values; in the muscle. The stimulating action of vildagliptin and PKF-275-055 on GLUT2 and GLUT4 expression, mRNA or protein, could be a mechanism by which, at least in part, the DPP-IV inhibitors exerts its lowering effect on blood glucose.

Sterol-regulatory-element-binding proteins (SREBPs) regulate the transcription of genes involved in cholesterol and fatty acid metabolism (Gang et al., 2007). The effects of insulin on SREBP-1c have been corroborated by in vivo studies showing that SREBP-1c expression and nuclear abundance were low in the liver of streptozotocin-induced diabetic rats, and markedly increased after insulin treatment (Valerio et al., 2006). The stimulating action of vildagliptin and PKF-275-055 on SREBP-1c mRNA concentration might be due insulin secretogogue effect; thereby decrease insulin resistance.

One of the key mechanisms in the pathogenesis of diabetes-related vascular dysfunction is an Oxidative stress. Oxidative stress is attributable to excessive production of reactive oxygen species (ROS) and inflammatory marker tumor necrosis factor-alpha (TNF-alpha). TNF-alpha is reported to downregulate eNOS expression and upregulate iNOS expression in rodents (Valerio et al., 2006; Hanrui et al., 2009). In this study, we observed the anti-inflammatory effect of vildagliptin and PKF-275-055.

It is believed that the islet insulin release depend on an intact production of nitric oxide (NO) within the islet vasculature (Moldovan et al., 2002; Svensson et al., 1994). Furthermore, L-arginine deficiency is associated with insulinopenia and a failure to secrete insulin in response to glucose. These authors demonstrated that sodium nitroprusside increased cGMP in rat islets and stimulated insulin secretion. In contrast, the present study demonstrated that diabetic rats showed significant increase in NO and decrease in insulin secretions and vice-versa in normal rats. Both vildagliptin and PKF-