Need Of Present Investigation
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Glucagon-like peptide 1 (GLP-1) is a gut-derived hormone shown to enhance glucose-dependent insulin secretion, suppress inappropriately high glucagon secretion, slow gastric emptying, and reduce food intake (Drucker, 2006). In some type 2 diabetic patients, GLP-1 levels are reduced, and elevation of GLP-1 by continuous infusion of the peptide leads to reductions in fasting glucose, postprandial glucose excursions, and glycated hemoglobin because it is rapidly inactivated by dipeptidyl peptidase-4 (DPP-4) (Deacon et al., 1995; Vilsboll et al., 2003).

Vildagliptin is an orally effective selective DPP-4 inhibitor. In diabetic patients, vildagliptin improved glycemic control, increased the plasma insulin–to–glucagon molar ratio, and reduced glycated hemoglobin levels (Ahren et al., 2004; Ahren et al., 2004). Short half life (2.8 hrs); inefficient inhibition of gastrointestinal functions, and diabetic complications with the administration of vildagliptin has always been a matter of concern (He et al., 2009; Adrian et al., 2007). Hence it was imperative to search for new long acting DPP-4 analogues. The discovery of PKF-275-055, analogue of Vildagliptin was in persuasion of the unmet need.

Recent studies have suggested that exogenous GLP-1 or GLP-1 derivatives cause a delay in gastric emptying both in healthy volunteers and type 2 diabetes patients (Nauck et al., 1997; Wettergren et al., 1993; Delgado et al., 2002). On the other hand, no delay in gastric emptying occurred when the endogenous GLP-1 level increased following administration of DPP-IV inhibitors (Balkan et al., 1999). One possible explanation for this discrepancy is that there may be a difference in the plasma GLP-1 levels that induce the incretin and gastrointestinal effects. Therefore, it is important to investigate the effect of DPP-IV inhibitors on gastrointestinal functions and plasma GLP-1 levels.

The facilitated transport of glucose across the plasma membrane of mammalian cells is catalyzed by a family of glucose transport proteins (GLUTs). Dysregulation of glucose transporter isoforms GLUT2 (liver) and GLUT4 (muscle) controlling mechanism can result in the pathophysiologic states associated with diabetes. In type 2 diabetic rats, GLP-1 treatment seems to act in liver GLUT2 and muscle GLUT4 expression exerted at the transcriptional as well as translational level (María et al., 2001). GLP-1 also increases
glycogen synthase activity, glycogen synthesis and glucose oxidation and utilization (Alcántara et al., 1997; Ruiz et al., 1992; Perea et al., 1997).

Under euglycemic conditions, insulin-stimulated muscle glucose uptake is enhanced in chow-fed Glp1r knockout (Glp1r<sup>−/−</sup>) mice. In light of this finding, it is paradoxical that suppression of endogenous glucose appearance by insulin is impaired in Glp1r<sup>−/−</sup> mice (Brown and Goldstein, 1997). This suggests a role for the Glp1r to regulate the balance of glucose disposal between the liver and muscle by modulating insulin action in these tissues.

Sterol-regulatory-element-binding proteins 1c (SREBP1c) regulate the transcription of genes involved in cholesterol and fatty acid metabolism (Ayala et al., 2009). SREBP-1c expression and nuclear abundance were low in the liver of streptozotocin-induced diabetic rats, and markedly increased after insulin treatment (Shimomura et al., 1999). Downregulation of the GLP-1mRNA, SREBP1c mRNA, and GLUT-4 mRNA expressions found in diabetes (Ruiz et al., 1992; Shimomura et al., 1999; Gang et al., 2007).

Oxidative stress is known to be the key mechanism in the pathogenesis of diabetes-related vascular dysfunction. 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. Nitric oxide (NO) is generated from L-arginine in inflamed tissues by iNOS. Under the stimulus of cytokines, invading macrophages and β -cells themselves may produce large amount of NO, leading to β-cells dysfunction and death (Valerio et al., 2006; Hao et al., 2006).

The aim of the present study was to determine whether long-acting vildagliptin analogue PKF-275-055 would favor regeneration of β-cell mass in n2-STZ rat model of diabetes mellitus. GLP-1 is secreted by the enteroendocrine L-cells in response to fat, meals, and carbohydrates (Kieffer et al., 1999). When acutely administered, it is known to enhance glucose-stimulated insulin release and glucose disposal in peripheral tissues, to suppress glucagon release and promote satiety, and to inhibit gastric emptying (Kieffer et al., 1999; Habener, 1993). More recently, it was demonstrated that GLP-1 and its analogs or the GLP-1 mimetic exendin-4 have demonstrated beneficial effects on increasing islet
neogenesis and differentiation as well as modulating β-cell mass in part by reducing apoptosis in animal models of diabetes (Yoon et al., 2008; Xu et al., 1999; Tourrel et al., 2002). These observations raises the question of what is the long-term impact of long acting DPP-IV inhibitor induced prolongation of GLP-1 on β-cell proliferation in terms of 1) β-cell mass enlargement, 2) improvement of the insulin resistance and β-cell function, 3) pancreatic cell apoptosis 4) improvement of glucose homeostasis and 5) gastrointestinal functions. To our knowledge, the answer to this important question has not been documented.

To address these issues, we investigated the capacity of vildagliptin and its analogue PKF-275-055 treatment to promote β-cell regeneration and thereby to improve islet function in the n2-STZ model of diabetes mellitus. Furthermore, in the current study, we investigated the role of chronic DPP-4 inhibition by a selective DPP-4 inhibitors vildagliptin and its analogue PKF-275-055 on glucose, insulin, HbA1c, GLP-1, NO, TNF-alpha, and glycogen levels in an n2-STZ model of diabetes mellitus. In this model, overt hyperglycemia results from a combination of insulin resistance and defects in insulin secretion induced by single dose STZ treatment. In addition, we have studied the effect of a prolonged treatment with PKF-275-055 on GLP-1 mRNA concentration in the pancreas, GLUT2 protein expression and SREBP1c mRNA concentration in the liver, and GLUT4 protein expression and mRNA concentration in skeletal muscle in n2-STZ model of diabetes mellitus.