Beneficial Effects of PKF275-055, a Novel, Selective, Orally Bioavailable, Long-Acting Dipeptidyl Peptidase IV Inhibitor in Streptozotocin-Induced Diabetic Peripheral Neuropathy


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ABSTRACT

1-[2-(adamantyl)amino]acetyl-2-cyano-(S)-pyrrolidine, monohydrochloride (PKF275–055), a vildagliptin analog, is a novel, selective, potent, orally bioavailable, and long-acting dipeptidyl peptidase IV inhibitor. We studied the effect of PKF275-055 administration on the prevention, protection, and treatment of diabetic neuropathy in the streptozotocin-induced diabetic rat. PKF275-055 improved body and muscle weight. Oral glucose tolerance tests showed a marked improvement in glucose metabolism under all treatment schedules. When tested in prevention and protection experiments, PKF275-055 completely averted the decrease of Na\(^+\)/K\(^+\)-ATPase activity and partially counteracted the nerve conduction velocity (NCV) deficit observed in untreated diabetic rats but had no effects on abnormal mechanical and thermal sensitivity. When used in a therapeutic setting, PKF275-055 induced a significant correction in the alteration in Na\(^+\)/K\(^+\)-ATPase activity and NCV present in untreated diabetics. Diabetic rats developed mechanical hyperalgesia within 2 weeks after streptozotocin injection and exhibited significantly longer thermal response latencies. It is noteworthy that PKF275-055 treatment restored mechanical sensitivity thresholds by approximately 50% (p < 0.01) and progressively improved the alteration in thermal responsiveness. In conclusion, PKF275-055 showed an anabolic effect, improved oral glucose tolerance, and counteracted the alterations in Na\(^+\)/K\(^+\)-ATPase activity, NCV, and nociceptive thresholds in diabetic rats. The present data support a potential therapeutic effect of PKF275-055 in the treatment of rodent diabetic neuropathy.

Introduction

Diabetic peripheral neuropathy (DPN) severely affects patients’ quality of life and contributes to morbidity and mortality (Vinik et al., 2000; Tesfaye 2009). Incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, are a family of peptides that potentiate insulin secretion after enteral ingestion of nutrients. These compounds have emerged as a new class of drugs for the treatment of type 2 diabetes (Baggio and Drucker, 2007). Their therapeutic potential, however, is limited by their rapid enzymatic inactivation in vivo by dipeptidyl-peptidase IV (DPP IV or CD26; EC 3.4.14.5) to biologically inactive metabolites. Inhibition of DPP IV, which boosts circulating GLP-1 levels, has proved useful in both patients with diabetes and animal models of type 2 diabetes (Pospisilik et al., 2003; Barnett 2006; McIntosh et al., 2006).

Vildagliptin, is a highly specific competitive and reversible inhibitor of DPP IV (Villhauer et al., 2003) that exhibits antidiabetic properties in both experimental paradigms and clinical trials (Burkey et al., 2005; Ahren and Foley 2008; Jin

ABBRévIATiONS: DPN, diabetic peripheral neuropathy; AUC, areas under the curve; DPP IV, dipeptidyl-peptidase IV; EDL, extensor digitorum longus; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; IENF, intraepidermal nerve fiber; NCV, nerve conduction velocity; OGTT, oral glucose tolerance test; STZ, streptozotocin; ANOVA, analysis of variance; CTRL, control; PKF275–055, 1-[2-(adamantyl)amino]acetyl-2-cyano-(S)-pyrrolidine, monohydrochloride.
Administration of vildagliptin to patients with type 2 diabetes and subjects with impaired glucose tolerance leads to a significant and persisting postmeal increase in active GLP-1, improves insulin secretion, ameliorates glycemic control, and reduces glucagon levels from baseline (Balas et al., 2007; Rosenstock et al., 2008). The safety and efficacy of vildagliptin has also been demonstrated (Ferrannini et al., 2009; Bosi, 2010). Vildagliptin administration raises circulating levels of GLP-1 and improves glucose tolerance in different animal models of insulin resistance, Zucker diabetic fatty rats, and streptozotocin (STZ)-induced diabetes in rat (Villhauer et al., 2003; Burkey et al., 2005; Jin et al., 2009). The STZ-induced diabetic rat, the most extensively studied animal model of type 1 diabetes, shares a number of features with human DPN. Although the decrease in nerve conduction velocity (NCV), along with the reduction of Na⁺-K⁺-ATPase activity, are the hallmarks of these rats, they also display various types of early neurological dysfunction (Coppey et al., 2000; Bianchi et al., 2004), including altered pain sensation, suggesting the involvement of small nociceptive sensory nerves (Lauria et al., 2003; Romanovsky et al., 2006).

Whereas chronic hyperglycemia is critical, insulin deficiency also contributes to the development of DPN (Pierson et al., 2003). Administration of trace amounts of insulin to STZ-diabetic rats can improve mitochondrial dysfunction in sensory neurons (Huang et al., 2003), myelinated sensory fiber atrophy (Brussee et al., 2004), epidermal unmyelinated sensory fiber loss and atrophy (Toth et al., 2006), and altered nociceptive responses (Romanovsky et al., 2006), without affecting blood glucose levels. Treatment of STZ-diabetic rats with a DPP-IV inhibitor showed marked improvements in glucose tolerance and fasting glucose levels and increased glucose-stimulated insulin secretion (Pospisilik et al., 2003). Perry et al. (2007) reported that GLP-1 has the ability to mediate neuroprotection in a rat model of peripheral sensory neuropathy induced by pyroxidine.

The rationale for the possible use of incretin-based agents in type 1 diabetes has been discussed in several reviews (Dupre, 2005; McIntosh et al., 2006; Bosi, 2010). These studies observed beneficial effects as reported previously in type 2 diabetes (Baggio and Drucker, 2007). Advantages of such a treatment might include the ability to protect β-cells from the action of proinflammatory cytokines and chemokines (Pospisilik et al., 2003; Blandino-Rosano et al., 2008). Recent studies showed that dorsal root ganglia, Schwann cells, and the peripheral nerve of diabetic rodents exhibit functional GLP-1R (Himeno et al., 2011; Jolivalt et al., 2011; Liu et al., 2011), suggesting that GLP-1R-mediated extracellular signal-regulated kinase signaling may prevent NCV slowing and intraepidermal nerve fiber (IEFN) loss (Jolivalt et al., 2011).

Those data are consistent with the beneficial effects of GLP-1 agonists, upon stimulation of GLP-1R, in models of Alzheimer’s disease, Parkinson’s disease (Harkavy and Whitton 2010), and polyneuropathy in diabetic mice and rats (Jin et al., 2009; Himeno et al., 2011; Liu et al., 2011). The neuroprotection of these agents is also effective in the IENF reduction observed in diabetic rodents (Jin et al., 2009; Himeno et al., 2011; Liu et al., 2011).

In the present study, we have investigated the effects of 1-[(2-adamantyl)amino]acetyl-2-cyano-(S)-pyrrolidine, monohydrochloride (PKF275–055), a vildagliptin analog, which is a novel, selective, potent, orally bioavailable, and long-acting DPP IV inhibitor (Villhauer et al., 2003) in STZ-induced diabetic rats. Our results indicate that PKF275-055 can improve nerve functions by preventing, blocking, or counteracting the progression of DPN in experimental diabetes.

Materials and Methods

Animals. The Statement of Compliance (Assurance) with Standards for Humane Care and Use of Laboratory Animals has been reviewed (10/28/2008) and approved by the National Institutes of Health–Office for Protection from Research Risks (5023-01, expiration 10/31/2013). The Institute for Pharmacological Research “Mario Negri” adheres to the principles set out in the following laws, regulations, and policies providing internal authorization for persons conducting animal experiments; the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996); and European Union directives and guidelines (Legislative Decree 626, September 19, 1994; 89/391/CEE, 89/654/CEE, 89/655/CEE, 89/656/CEE, 90/269/CEE, 90/270/CEE, 90/394/CEE, 90/679/CEE).

Male Sprague-Dawley rats weighing 180 to 200 g (Charles River Italia, Calco, Italy) were housed in groups of two. Room temperature and relative humidity were set at 22 ± 2°C and 55 ± 10%, respectively. Artificial lighting provided a 12-h light/dark cycle (7:00 AM to 7:00 PM). The animals had free access to dietary food and water.

Two studies were done in which we tested the efficacy of PKF275-055 administration in preventing or reversing nerve dysfunction in STZ-induced diabetes. Seventy two animals were included in the studies. At the beginning of studies, rats were randomly divided into the following groups: control (n = 16); control, PKF275-055, for prevention and therapeutic schedules (n = 16); STZ-untreated (n = 16); STZ- PKF275-055 for prevention (n = 8); STZ- PKF275-055 for protection (n = 8), and STZ- PKF275-055 for therapy (n = 8).

Compounds and Dosing. Vildagliptin belongs to the group of compounds with N-substituted glycyl-2-cyano pyrrolidine (1-[(3-hydroxy-1-adamantyl)amino]acetyl)-2-cyano-[S]-pyrrolidine). PKF275-055 (provided by Novartis, Basel, Switzerland) is the des-hydroxy analog of a regio-isomer of vildagliptin where PKF275-055 is a 2-aminoadamantane derivative (Villhauer et al., 2003).

Study 1 investigated the in vivo efficacy of PKF275-055 in preventing and/or protecting the development of peripheral diabetic neuropathy in the STZ-induced diabetes rat model. Study 2 examined the in vivo efficacy of PKF275-055 chronically administered in the treatment of established peripheral DN in STZ-diabetic rats. In study 2, four rats from the protective schedule group in study 1 continued the treatment for another 5 weeks. The flow chart of the studies is shown in Fig. 1.

For diabetes induction, overnight fasted rats received a single intraperitoneal injection of STZ (Sigma-Aldrich, St. Louis, MO), 60 mg/kg b.wt., in sodium citrate buffer, pH 4.5. Only rats with urine glucose >15 mM 2 days after STZ injection were used in the protection and therapeutic protocols. Control animals were age-matched and given sodium citrate buffer without STZ.

During these in vivo studies, PKF275-055 was administered orally by gavage by using 0.5% aqueous hydroxyethylcellulose as vehicle, at 3 mg/kg b.wt. for the first week in the prevention groups. Subsequently, it was administered in drinking water at the same dosage throughout the study for both the prevention and protective schedules. STZ was injected either 1 week or 2 days after PKF275-055 treatment for the prevention or protection studies, respectively, and observations lasted 5 weeks (Fig. 1). In the therapeutic schedule, treatment with the DDP IV inhibitor (as above) started 5 weeks after induction of diabetes and lasted 5 weeks, at a dose of 10 mg/kg b.wt.
Control animals received the same treatment regimen. Given that water intake increased greatly in STZ-diabetic rats, daily water consumption was measured every other day, and PKF275-055 concentration was adjusted to reach the indicated dosages.

**Analytical Procedures.** For each experimental protocol, water and food intake were measured daily (10:00 AM). Blood glucose, body weight (10:00 AM, under fed conditions), and mechanical and thermal nociceptive thresholds were recorded weekly. Na⁺,K⁺-ATPase activity in the sciatic nerves was assessed at the end of each experimental period. For measurement of plasma GLP-1 levels, blood samples were obtained from the tail vein under fasting and fed conditions, and GLP-1 was measured as described previously (Burry et al., 2005).

**Behavioral Evaluation.** The nociceptive threshold to radiant heat was quantified by using the hot-plate paw withdrawal test as described previously (Bianchi et al., 2004). In brief, a 40-cm-high Plexiglas cylinder was suspended over the hot plate, and the temperature was maintained at 50°C to give a latency of approximately 10 s for control rats. Withdrawal latency was defined as the time between placing the rat on the hot plate and the time of withdrawal, licking the hind paw, or manifesting discomfort.

Mechanical allodynia on the plantar surface of the rat was quantified, following the manufacturer’s directions, by the dynamic paw withdrawal test with a Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio, Italy), which generates a linearly increasing mechanical force. The actuator filament (0.5-mm diameter) produces force over the full range of typical aesthesiometer test devices. The paw withdrawal reflex is automatically recorded by using the latency until withdrawal, in seconds, and the force at which paw is withdrawn, in grams.

**Oral Glucose Tolerance Test.** Blood glucose was measured in fed animals, and an OGTT was performed on treatment days 35 and 78 in overnight (16 h)-fasted animals, after a glucose challenge of 1.25 mM EGTA, and 10 mM Tris, pH 7.5. Composite, Na⁺,K⁺-ATPase, and Mg⁺²-ATPase activities were determined spectrophotometrically at 340 nm (Ultrorpec 2100 pro; GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) by the coupled-enzyme assay, which continuously monitored NADH oxidation. Na⁺,K⁺-ATPase activity was defined as the ouabain-inhibitable activity (3 mM, final concentration). Protein content was determined with the DC Protein Microplate Assay Protocol (Bio-Rad Italy, Milan, Italy) according to the manufacturer’s specifications.

**Data Analysis.** Statistical differences were analyzed by one-way ANOVA, followed by the Tukey-Kramer post-test for group-wise comparisons, as specified in the figure legends.

**Results**

**Study 1**

**Effect of PKF275-055 on Changes in Body Weight, Plasma Glucose Levels, Food and Water Intake, and Oral Glucose Tolerance Test in STZ-Induced Diabetic Rats.** Table 1 shows that, on average, STZ-induced diabetic rats gained less weight than their age-matched controls over the 5 weeks of this study. Administration of PKF275-055 either under the prevention or the protection protocols had no significant effects on weight gain in diabetic rats or controls. At the beginning of the study, diabetic rats were randomized for blood glucose levels to eliminate differences among these groups. At sacrifice nonfasted plasma glucose levels were four to five times higher in diabetic groups, regardless of whether they were treated with PKF275-055 or not (Table 1). As expected, there was a significant difference in food and water intake between diabetic and control rats. Administration of the DDP inhibitor under either protocol did not modify this difference (Table 1).

OGTTs showed that PKF275-055 reduced the glucose swings (Table 1). The effect of PKF275-055 on glucose AUC was statistically significant, with a complete normalization and partial reversal for protection and prevention groups, respectively, compared with untreated, STZ-induced diabetic rats.

**PKF275-055 Administration Prevents and Protects Diabetes-Induced Changes in Nerve Na⁺,K⁺-ATPase Activity.** Diabetes reduced Na⁺,K⁺-ATPase activity in sciatic nerve by approximately 40%, from 0.66 ± 0.02 in nondiabetic animals.
abietic rats to 0.42 ± 0.07 in the untreated STZ-induced diabetic group (p < 0.005). It is noteworthy that diabetic rats treated with PKF275-055 under either the prevention or protection schedules exhibited only a slight reduction in Na⁺,K⁺-ATPase activity that was significantly different from that in control rats (0.54 ± 0.12 and 0.52 ± 0.09 for protection and preventive groups, respectively). Minor Effects of PKF275-055 on Alterations in Thermal and Mechanical Nociceptive Thresholds in Experimental Diabetes. As expected, STZ treatment significantly increased the hindpaw thermal withdrawal latency from week 2 to week 5 (23.4 ± 1.9 and 15.7 ± 0.9 s in untreated diabetic and control rats, respectively). PKF275-055 did not affect the response latencies in nondiabetic rats at any time and triggered only a relatively minor, nonsignificant decrease in latency when it was administered under either the prevention or the protection schedules (-9 and -29%, respectively, compared with the untreated STZ-induced diabetic group).

The mechanical paw force withdrawal threshold (mechanical allodynia) in diabetic rats was lower at all times than for controls (15–20%), and PKF275-055 did not improve this alteration.

Effects of Diabetes and PKF275-055 on Electrophysiological Parameters. Five weeks after STZ injection the reduction in NCV in the diabetic group (-44%; from 32.1 ± 0.25 m/s of the untreated STZ-induced group, mean core temperature 36.5 ± 0.12°C) was only partially improved by 11 and 15% in rats subjected to PKF275-055 prevention and protection protocols, respectively (mean core temperature 36.1 ± 0.33 and 36.3 ± 0.22°C, respectively).

Study 2

Effect of PKF275-055 Effect on Changes in Body and Muscle Weight, Plasma Glucose Levels, Na⁺,K⁺-ATPase Activity, and Food and Water Intake in Experimentally Diabetic Rats. Body and muscle weight and changes in plasma glucose levels and Na⁺,K⁺-ATPase activity during the experimental period are shown in Fig. 2 and Table 2. STZ-induced diabetic rats on average gained less weight than age-matched controls throughout the study period (Fig. 2). PKF275-055 (10 mg/kg b.wt.) under the therapeutic schedule, starting 5 weeks after STZ injection, had no significant effects on weight gain in control rats. We observed a slight body weight recovery in PKF275-055-treated diabetic rats under the therapeutic schedule and also for the group treated with 3 mg/kg PKF275-055 during the whole period (prolonged prevention group, 10 weeks; Fig. 2). To better evaluate these changes, we weighed two leg skeletal muscles, the soleus, a prevalently red-fiber muscle, and the extensor digitorum longus (EDL), a prevalently white fiber muscle. As presented in Table 2, the soleus was less affected by the diabetic state than the EDL (51 and 67% reduction, respectively). PKF275-055 restored EDL weight by 39% in the therapeutic group and 48% in the prolonged preventive groups. These observations were confirmed when muscle weight was normalized by body weight (Table 2).

At the end of the experimental period, both nonfasted and overnight-fasting plasma glucose levels had increased 4- to 5-fold in diabetic rats. In contrast, diabetic rats that received PKF275-055 exhibited blood glucose levels similar to those in untreated diabetic rats (data not shown).

It is noteworthy that the PKF275-055 therapeutic and prolong prevention schedules completely normalized and improved, respectively, the reduction in sciatic nerve Na⁺,K⁺-ATPase activity in diabetics.

In study 2, we also observed that the significant increases in food and water intake associated with the diabetic state (food intake: 26.8 ± 0.5 and 44.8 ± 1.0 g in control and untreated STZ group; water intake: 32.5 ± 1.1 and 204.7 ± 11.5 ml for the same groups) were not modified after PKF275-055 administration under both drug protocols (data not shown).

Effects of PKF275-055 on Alteration in Oral Glucose Tolerance Triggered by Diabetes. Although PKF275-055 had no significant effect on fasting plasma glucose levels in control rats after 5 weeks, a 10 mg/kg daily dose triggered a
significant improvement of this parameter in diabetic rats (Fig. 3, inset).

OGTTs at the end of treatment showed that diabetic rats had impaired glucose metabolism characterized by a doubling in AUC compared with their control counterparts. PKF275-055 administration did not alter glucose responses during the 120-min course of OGTT in control rats but significantly reduced by 46% the glucose swings in diabetic-treated rats (Fig. 3).

Effects of PKF275-055 on Changes in Mechanical and Thermal Nociceptive Thresholds Associated with Experimental Diabetes. Figure 4A shows the hind paw thermal withdrawal thresholds measured over the 5-week period of PKF275-055 treatment under the therapeutic schedule. Five weeks after STZ injection the thermal response latency was significantly increased. It is noteworthy that this hypalgesia was reversed by PKF275-055 administration, with this effect reaching statistical significance after 3 weeks of treatment (Fig. 4A). In contrast, PKF275-055 did not affect the latencies in controls. Figure 4B presents the hind paw force withdrawal thresholds (mechanical allodynia) during the therapeutic protocol. Diabetic rats showed a decrease in the mechanical thresholds at all times. Starting from a comparable alldynic situation (~20%), PKF275-055 progressively and significantly improved the mechanical thresholds, reaching a 11.6% reduction from controls at the end of treatment.

Effects of PKF275-055 on Alterations in Nerve Conduction Velocity in Diabetic Rats. Five weeks after STZ injection (i.e., at randomization immediately before the beginning of treatment) NCV was significantly reduced \((p < 0.001)\) in STZ compared with control groups (Fig. 5). Administration of PKF275-055 under the therapeutic schedule triggered a 50% improvement in NCV in diabetic rats but did not affect this parameter in their control counterparts (Fig. 5).

Effect of PKF275-055 on GLP-1 Levels. We measured plasmatic GLP-1 levels in rats subjected to long-term treatment with PKF275-055 (10 mg/kg b.wt.), fasted and 2 and 4 h after glucose loading. As expected, we measured a significant reduction of GLP-1 in the diabetic group in fasting conditions, from 9.8 ± 0.27 pM in nondiabetic rats to 2.5 ± 0.57 pM in the untreated STZ-induced diabetic group. We also observed a significant increase of GLP-1 in the PKF275-055-treated fasted rats (16.7 ± 2.10 pM). At 2 and 4 h after glucose loading, a similar significant trend was observed in the PKF275-055-treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle Weight</th>
<th>Body Weight/Muscle Weight</th>
<th>Na⁺,K⁺-ATPase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOL mg</td>
<td>EDL mg</td>
<td>SOL g/mg</td>
</tr>
<tr>
<td>Control</td>
<td>204 ± 5.7</td>
<td>221 ± 7.0</td>
<td>2.48 ± 0.08</td>
</tr>
<tr>
<td>Control + PKF</td>
<td>210 ± 4.2</td>
<td>227 ± 4.3</td>
<td>2.43 ± 0.08</td>
</tr>
<tr>
<td>STZ untreated</td>
<td>99 ± 9.8</td>
<td>73 ± 8.7**</td>
<td>2.47 ± 0.07</td>
</tr>
<tr>
<td>STZ + PKF therapeutic</td>
<td>102 ± 3.7*</td>
<td>95 ± 4.6**§</td>
<td>2.63 ± 0.08</td>
</tr>
<tr>
<td>STZ + PKF prolong prevention</td>
<td>122 ± 6.6**</td>
<td>108 ± 4.5**§</td>
<td>2.43 ± 0.03</td>
</tr>
</tbody>
</table>

* \(p < 0.001\) vs. CTRL and CTRL + PKF.
** \(p < 0.005\) vs. CTRL and CTRL + PKF.
*** \(p < 0.01\) vs. CTRL and CTRL + PKF.
§ \(p < 0.05\) vs. STZ untreated.

TABLE 2
Soleus and EDL weights and Na⁺,K⁺-ATPase activity in control, diabetic (STZ), and PKF275–055-treated diabetic rats subjected to therapeutic or prolong prevention schedules at day 78 after STZ injection

Results are means ± S.E.M.

Fig. 3. OGTTs in overnight fasted rats on day 78 after PKF275-055 administration (PKF) according to study 2 (see Materials and Methods). Reactive glucose AUC and blood glucose measured during the OGTT (inset) are shown. ****, \(p < 0.001\) versus CTRL and CTRL + PKF; §§, \(p < 0.01\) versus STZ untreated; §, \(p < 0.05\) versus STZ untreated.
Much interest has been focused on the potential therapeutic effects of incretins in type 2 diabetes. In addition to their action on insulin secretion and glucose regulation, the growing list of their effects offers a rationale for investigating their efficacy in type 1 diabetes (Dupre, 2005; Lovshin and Drucker, 2009; Bosi, 2010).

We tested whether long-term DPP IV inhibition, using the potent vildagliptin analog PKF275-055, would be beneficial in experimental DPN. In study 1 we tested the possible efficacy of PKF275-055 at preventing and/or protecting the development of DPN. We observed normalization of \( \text{Na}^+/\text{H}^- \)-ATPase activity by PKF275-055, along with a partial recovery of the NCV deficit and a small improvement in nociception. These partial effects may arise from the use of a low dose of inhibitor or a short period of treatment. In study 2 we found that, regardless of any glucose-lowering effect, chronic treatment of diabetic animals with a higher dose of PKF275-055 improved anabolic function, restored NCV and \( \text{Na}^+/\text{K}^+ \)-ATPase activity deficits, and impaired nociceptive thresholds. These beneficial effects were observed in the early phase of metabolic derangement of diabetes, when changes in \( \text{Na}^+/\text{K}^+ \)-ATPase activity and NCV, as well as markers of painful neuropathy, are present. However, additional studies in long-term diabetes will be required to evaluate the effect of prolonged DPP-IV inhibition on late structural changes.

Changes in body weight and water and food intake provide an estimate of the overall metabolic derangement. In the present study, treatment with PKF275-055, as shown previously with vildagliptin in Zucker rats and the STZ diabetic...
model, did not significantly boost water and food intake (Burkey et al., 2005; Jin et al., 2009; B. F. Burkey, unpublished observation). Although not significant, we observed a tendency toward body weight gain in rats subjected to the prevention and protection protocols and a similar trend in the therapeutic and prolong prevention protocols (Table 1). To better analyze benefits of this drug on the anabolic function we also examined the weight of red (soleus) and white (EDL) leg muscles in study 2. These muscles were affected differently by diabetes, the soleus being less affected than the EDL. Of interest, PKF275-055 caused a partial, but significant, reversal of the STZ-induced changes in EDL weight (Table 2). These results suggest an improvement of the overall metabolic state and/or selective changes in the state of denervation/innervation of the two different muscles.

Several reasons may underlie the beneficial effect DPP IV inhibition in type 1 diabetes. PKF275-055 did not affect blood glucose, but it is likely to inhibit circulating DPP IV activity, which, in turn, improves GLP-1 secretion. This is similar to the effects reported for its parent compound vildagliptin in chronically treated Zucker rats (Burkey et al., 2005). We also obtained preliminary data showing an increase in GLP-1 plasma levels in long-term PKF275-055-treated, STZ-induced diabetic rats, both in fasting and after glucose loading conditions. Recent studies show that GLP-1 agonists can ameliorate neuropathy in diabetic rats and mice (Himeno et al., 2011; Jolivalt et al., 2011; Liu et al., 2011). In this regard, several studies have shown increased GLP-1 levels after administration of the DDP-IV inhibitor alagliptin in a non-obese model of type 2 diabetes (Asakawa et al., 2009) and vildagliptin in STZ-induced type 1 diabetes (Jin et al., 2009; B. F. Burkey, unpublished observations). Therefore, although we do not have direct data, we suggest that higher GLP-1 levels in incretin-treated rats might be beneficial in diabetic neuropathy and possibly lead to reduced glucose excursion after OGTT. It is important to note that the OGTT was performed 16 h after PKF275-055 withdrawal, thus measuring the cumulative effects of long-term DPP IV inhibition as opposed to an acute incretin increase (Table 1; Fig. 3).

Jin et al., (2009) reported that treatment with vildagliptin (0.3 and 10 mg/kg) for 32 weeks partially improves the reduction in insulin levels in STZ-diabetic rats. This occurs in spite of unaffected fasting glucose and glycated hemoglobin (HbA1c) levels but is associated with an increase in GLP-1 plasma levels. We postulate that a similar increase in our experimental conditions may account for the observed beneficial effects as supported by OGTT data.

The effects of DDP IV inhibition on immune functions include suppression of T-lymphocyte proliferation, T-cell stimulation of B-lymphocyte immunoglobulin release, T helper 1 cytokine production, and transendothelial migration of T-cells (Aytaç and Dang 2004). An additional advantage is provided by GLP-1, which may be able to protect β-cells from proinflammatory cytokines (Blandino-Rosano et al., 2008). Although the STZ-diabetic model does not have a strong autoimmune component, islets have a rapid apoptotic phase that might be responsible for islet destruction in human type 1 diabetes. Consequently, DPP IV inhibition could have an additional effect on type-1 diabetes: potentiation of glucose-dependent insulinotropic polypeptide and GLP-1 responses may reduce apoptosis and stimulate β-cell neogenesis and growth, resulting in the preservation of β-cell mass at disease onset when a discrete mass of β cells is still present (Harkavyi and Whitton 2010).

Low Na⁺,K⁺-ATPase activity in peripheral nerves may explain the slowing of NCV during hyperglycemia. The reduction of this activity in patients with diabetes is not simply secondary to fiber loss but, quite likely, contributes to the pathogenesis and self-maintenance of diabetic neuropathy (Scarpini et al., 1993). Na⁺,K⁺-ATPase is sensitive to environmental hypoxia and oxidative stress (Doss et al., 1997), and pharmacological treatments to prevent or restore this activity are able to protect or reverse the decrease in NCV (Bianchi et al., 2004). The present study demonstrates that PKF275-055 counteracts the impairment of Na⁺,K⁺-ATPase activity in diabetic rats, suggesting that this mechanism might be responsible for the improvement in neural function driven by this DPP-IV inhibitor, as assessed by NCV. The effect of PKF275-055 on Na⁺,K⁺-ATPase activity may de-
pend on preventing changes in its subcellular localization, the expression or phosphorylation/glutathionylation/oxidation of the different enzyme subunits, or axon Na⁺-permeability, all of which are critical for its enzymatic activity.

Another aim of this study was to assess whether continuously administered PKP275-055 helped relieve neuropathic pain in experimental DPN. Under the therapeutic protocol, this inhibitor significantly ameliorates diabetic pain, as shown by the prolonged withdrawal latencies to paw pressure and shortening of tolerance to thermal stimuli (Fig. 4). At randomization, before the onset of the therapeutic protocol (day 35 after STZ), diabetic rats displayed a significant increase in thermal nociceptor latency and a reduction in mechanical thresholds. Our data confirm previous results showing that STZ-induced diabetes in rats is associated with mechanical hyperalgesia (Malcangio and Tomlinson, 1998; Bianchi et al., 2004; Sugimoto et al., 2008). In contrast, conflicting data have been reported regarding thermal perception in experimental DPN, including decreases (Courteix et al., 1993) or increases (Bianchi et al., 2004; Sugimoto et al., 2008; Jolivalt et al., 2011) in the thermal nociceptive thresholds of STZ-induced diabetic rodents. Alterations in nociceptive thresholds can be partially prevented and restored by erythropoietin, neuroactive steroids, prosaposin-derived peptide, sulfosalazine, and GLP-1 agonists (Calcutt et al., 2000; Bianchi et al., 2004; Berti-Mattera et al., 2008; Roglio et al., 2008; Jolivalt et al., 2011). The nociceptive dysfunction arising immediately after the onset of diabetes may be concurrent with the rapid impairment of peripheral nerve insulin receptor signaling. (Sugimoto et al., 2008). There are clear indications that in the STZ-induced diabetic rat model trace amounts of insulin, administered exogenously would be beneficial on nociceptive responses without affecting blood glucose (Huang et al., 2003; Brussee et al., 2004; Romanovsky et al., 2006). Consequently, the peripheral nerve, a critical site of diabetic complications, seems to be insulin-responsive, exhibiting a range of early functional changes during insulin deficiency. Our data on the nociceptive effects of PKP275-055 correlate well with those of Jin et al., (2009) who observed that treatment of STZ-diabetic rats with a DPP-IV inhibitor improves sensory thresholds to pressure, vibration, and pain or temperature and rescue IENF loss with a concomitant increase in GLP-1 levels. These data are in agreement with others showing that GLP-1 signals might prevent NCV slowing and pain IENF loss in STZ-diabetic rodents (Himeno et al., 2011; Jolivalt et al., 2011; Liu et al., 2011).

Another study described impaired peripheral nerve insulin receptor signaling during the early course of nociceptive dysfunction in STZ-diabetic rats (Sugimoto et al., 2008). We speculate that PKP275-055 may improve nociceptive responses in STZ diabetic rats through the rise in insulin release and other effects mediated by GLP-1.

In summary, the present findings are consistent with the usefulness of PKP275-055 as an effective strategy for preventing and relieving symptoms of peripheral diabetic neuropathy in rodents.

Authorship Contributions

Participated in research design: Bianchi, Burkey, Ghezzi, and Cavaletti.

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References


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