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A novel dipeptidyl peptidase-4 inhibitor, alogliptin (SYR-322), is effective in diabetic rats with sulfonylurea-induced secondary failure

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ABSTRACT

Aims: Loss of efficacy over time or secondary failure occurs somewhat often and remains a major concern of sulfonylurea (SU) therapy. In this study, we investigated the benefits of alogliptin, an oral, potent and highly selective dipeptidyl peptidase-4 (DPP-4) inhibitor, in a rat model exhibiting SU secondary failure.
Main methods: Neonatally streptozotocin-induced diabetic rats (N-STZ-1.5 rats), a non-obese model of type 2 diabetes, were used in these studies. The effects of alogliptin on DPP-4 activity and glucagon-like peptide 1 (GLP-1) concentration were determined by measuring their levels in plasma. In addition, the effects of alogliptin on an oral glucose tolerance test were investigated by using an SU secondary failure model.
Key findings: Alogliptin dose dependently suppressed plasma DPP-4 activity leading to an increase in the plasma active form of GLP-1 and improved glucose excursion in N-STZ-1.5 rats. Repeated administration of glibenclamide resulted in unresponsiveness or loss of glucose tolerance typical of secondary failure. In these rats, alogliptin exhibited significant improvement of glucose excursion with significant increase in insulin secretion. By contrast, glibenclamide and nateglinide had no effect on the glucose tolerance of these rats.
Significance: The above findings suggest that alogliptin was effective at improving glucose tolerance and therefore overcoming SU induced secondary failure in N-STZ-1.5 rats.

Introduction

Type 2 diabetes mellitus is a progressive disease primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia. Sulfonylureas (SUs) have been widely used either as monotherapy or in conjunction with another class of antidiabetic medications. These agents stimulate insulin release from pancreatic β-cells via inhibition of the ATP-sensitive potassium (K_{ATP}) channel and are typically used as first-line treatment. However, after long-term SU therapy, sometimes as little as six months, a number of patients begin to show an increase in their blood glucose levels (Matthews et al. 1998), necessitating the addition of another drug class with a different but complementary mechanism of action. This clinical phenomenon has been termed SU secondary failure, and is thought to be a result of the progressive loss of β-cell function over the course of type 2 diabetes (Riedel et al. 2007).

Glucagon-like peptide-1 (GLP-1) is one of the most potent insulinotropic hormones; it prevents gastric emptying, glucagon secretion, and moderates food intake as well as showing impressive antidiabetic actions (Drucker 1998; Nauck 1999; Wang et al. 1995). Furthermore, it has been reported that GLP-1 stimulates regeneration and differentiation of pancreatic β-cells (Hui et al. 2001). Exogenous GLP-1 treatment effectively lowers plasma glucose concentration in patients with advanced type 2 diabetes long after SU secondary failure has occurred (Nauck et al. 1998). It is likely that patients respond differently to SU and GLP-1 since their mechanisms of action differ. The former class of drugs directly induces a closure of the potassium channel and a depolarization of β-cells, which then results in a subsequent increase in intracellular calcium concentrations. The latter hormone mainly acts by augmenting cyclic AMP synthesis.

GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) (Kieffer et al. 1995; Deacon et al. 1995) to an inactive form; therefore, prevention of its inactivation by inhibiting DPP-4 activity is expected to enhance the antidiabetic effects of GLP-1. Alogliptin is a novel, potent and highly selective DPP-4 inhibitor that was shown to effectively lower plasma glucose by stimulating insulin secretion in a glucose tolerance test with female Wistar fatty rats (Feng et al. 2007). Since the glucose-lowering effect of DPP-4 inhibitors is based on their inhibition of GLP-1 degradation, a mechanism different from SUs, alogliptin may be effective in the treatment of type 2 diabetic patients who exhibit SU secondary failure.

In the present study, we measured active GLP-1 concentrations in plasma of neonatally streptozotocin-induced diabetic rats (N-STZ-1.5 rats), a model of non-obese diabetes, to confirm that insulinotropic and

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glucose-lowering effects of alogliptin were attributable to inhibition of DPP-4. An oral glucose tolerance test was performed to examine the glucose-lowering activity of alogliptin in the N-STZ-1.5 rats. Furthermore, a rat model of SU secondary failure was generated by using N-STZ-1.5 rats. This model was used to determine the efficacy of alogliptin in treating type 2 diabetes or maintaining glycemic control in the N-STZ-1.5 rats with SU secondary failure.

Materials and methods

Chemicals

Alogliptin was obtained from Takeda San Diego, Inc. Glibenclamide was purchased from Wako, Japan. Nateglinide was extracted from tablets (Starsis®, Yamanouchi Pharmaceutical Co. Ltd., Japan) at the Medicinal Chemistry Research Laboratories, Takeda Pharmaceutical Company Limited.

Animals

The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company, Ltd (Osaka, Japan).

Male N-STZ-1.5 rats were obtained from Takeda Rabics, Ltd (Osaka, Japan). The N-STZ-1.5 rats were prepared by subcutaneous injection of 120 mg/kg of streptozotocin (Sigma, MO, USA) in male Wistar Kyoto rats at 1.5 days after birth. They were fed with standard laboratory rodent chow CE-2 (CLEA Japan, Inc., Tokyo, Japan) and tap water ad libitum.

Active GLP-1 levels and DPP-4 activity in plasma of N-STZ-1.5 rats

At 41-weeks of age, male N-STZ-1.5 rats were fasted overnight and divided into five groups ($n=8$ for each group); each group was given vehicle (0.5% methylcellulose) or alogliptin at 0.1, 0.3, 1 or 3 mg/kg, p.o. Ninety minutes after drug or vehicle administration, blood samples were collected from the tail vein to determine baseline activity of plasma DPP-4. Next, 2 h post dosing; all animals received a liquid meal (F2LCP, 20 kcal/kg, Oriental Yeast Co., Ltd., Tokyo, Japan) orally via gavage. Blood samples were collected from the tail vein 5 min after the liquid meal load for measurement of plasma active GLP-1.

To measure plasma DPP-4 activity, 20 µL of plasma was mixed with 80 µL of assay buffer (0.25 mol/L Tris–HCl pH 7.5, 0.25% (w/v) bovine serum albumin, 0.125% (w/v) CHAPS) and the reaction was initiated by adding 100 µL of 1 mmol/L of Gly-Pro-pNA·Tos (Peptide Institute, Osaka, Japan) in distilled water as a substrate. The samples were incubated at 30 °C and the increase in absorbance at 405 nm was monitored at 20 and 60 min after reaction initiation. The relative activity of DPP-4 was assessed by the change in absorbance at 405 nm between 20 and 60 min. Active GLP-1 levels were measured by a two-site enzyme immunoassay (LINCO Research Inc., MO, USA).

Effect of a single dose of alogliptin on oral glucose tolerance test (OGTT) in N-STZ rats

Prior to the start of the study, 23-week old N-STZ-1.5 rats were fasted overnight and divided into six groups based on plasma glucose, glycosylated hemoglobin (GHb), and body weight ($n=6$ rats per group). Each group was administered vehicle (0.5% methylcellulose) or alogliptin at 0.03, 0.1, 0.3, 1 or 3 mg/kg p.o. One hour after drug or vehicle administration, all animals received an oral glucose load (1 g/kg). Blood samples were collected via the tail vein before the glucose load (time 0), and 10, 30, 60 and 120 min after the glucose load and plasma glucose and plasma immuno-reactive insulin (IRI) were determined.

SU secondary failure model of N-STZ-1.5 rats

To investigate the effect of alogliptin on the SU secondary failure, an N-STZ-1.5 rat model of SU secondary failure using glibenclamide was created. N-STZ-1.5 rats were divided into 2 groups ($n=12$ and 24). At 20 weeks of age, one group ($n=12$) received vehicle (0.5% methylcellulose) as the control group, while the other group ($n=24$) received glibenclamide at a dose of 10 mg/kg orally once daily for 27 days, as the secondary failure group. After 27 days of treatment with vehicle or glibenclamide, the rats receiving vehicle were divided into 2 groups ($n=6$ per group) and given either vehicle (0.5% methylcellulose) or glibenclamide orally, while the rats given glibenclamide were divided into 4 groups ($n=6$ per group) and administered vehicle (0.5% methylcellulose), glibenclamide (10 mg/kg), alogliptin (1 mg/kg) or nateglinide (50 mg/kg) by the oral route. One hour (vehicle, glibenclamide and alogliptin) or 0.5 h (nateglinide) after dosing, all animals received an oral glucose load (1 g/kg). Blood samples were collected via the tail vein prior to the glucose load (time 0) and 10, 30, 60, and 120 min post glucose load and plasma glucose and IRI levels were measured.

For measurement of pancreatic insulin content, five N-STZ-1.5 rats received vehicle for 4 weeks, and four N-STZ-1.5 rats received 10 mg/kg of glibenclamide for 4 weeks. Pancreata of the rats were homogenized and centrifuged in an acid-alcohol solution (0.15 N HCl in 75% ethanol) and the IRI levels of the supernatant were measured.

Measurements

Plasma glucose was measured enzymatically by Autoanalyzer 7080 (Hitachi, Japan). Blood glucose was measured enzymatically by Precision Q.I.D (Abbott, Japan). IRI was measured by using RIA (LINCO Research, MO, USA). GHb was measured by HPLC-based method using automated GHb Analyzer HLC-723 GHb V A1c2.2 (TOSOH, Japan). Plasma concentrations of intact GLP-1 were determined by enzyme-linked immunosorbent assay (ELISA, Glucagon Like Peptide-1 (Active) ELISA Kit, LINCO Research).

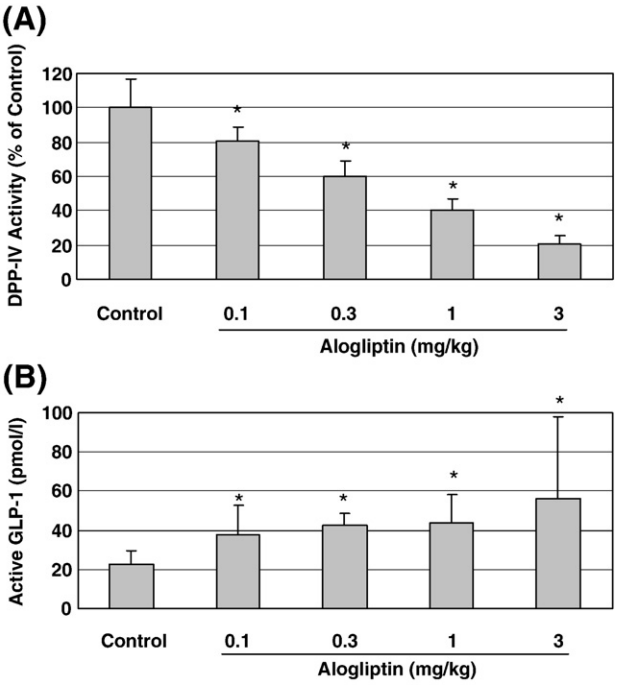

Fig. 1. Effect of alogliptin on plasma DPP-IV activity (A) and plasma active GLP-1 levels (B) in male N-STZ-1.5 rats. Values are mean and SD ($n=8$). All doses were able to significantly inhibit plasma DPP-4 activity. In addition, all doses were able to significantly increase the plasma active GLP-1 levels in male N-STZ-1.5 rats. *$p \leq 0.025$ vs. control by one-tailed Williams test.

Statistics

All results are described by mean values ± S.D. A one-tailed Williams' test was used to determine statistical difference between control and alogliptin on active GLP-1 concentrations and DPP-4 activity in plasma. In addition, a one-tailed Williams' test was used to determine statistical significance between a single dose of alogliptin or control on OGTT. A Student's *t*-test or Aspin–Welch test was used to determine statistical differences versus control and glibenclamide, alogliptin, and nateglinide on OGTT of N-STZ-1.5 rats with SU secondary failure.

Results

The effect of acute administration of alogliptin on plasma DPP-4 activity and active GLP-1 levels in N-STZ-1.5 rats

Alogliptin was administered to fasted N-STZ-1.5 rats at four different doses (0.1, 0.3, 1.0, and 3.0 mg/kg) as well as a control, and the effects on DPP-4 activity and active GLP-1 after a meal load were determined. As shown in Fig. 1A, plasma DPP-4 activity was significantly decreased ($p \leq 0.025$) in a dose-dependent manner by 20, 40, 60, and 79%, respectively, in the 0.1, 0.3, 1.0, and 3.0 mg/kg alogliptin dosing groups when compared to the vehicle treated group. In correlation with the DPP-4 data, a significant ($p \leq 0.025$) dose-dependent increase in active GLP-1 was observed when compared to the vehicle treated group (Fig. 1B). Active GLP-1 concentrations were increased by 1.7- 1.9-, 2.0-, and 2.5-fold, respectively, in the 0.1, 0.3, 1.0, and 3.0 mg/kg alogliptin dosing groups compared to the vehicle treated group.

Glucose-lowering effects of alogliptin in N-STZ-1.5 rats

To identify the antidiabetic effects of alogliptin in N-STZ-1.5 rats, an oral glucose tolerance test was performed after the administration of alogliptin (0.03, 0.1, 0.3, 1.0 or 3.0 mg/kg) or vehicle. As shown in Fig. 2A and C, oral administration of alogliptin in N-STZ-1.5 rats produced dose-dependent improvements in glucose tolerance with a minimum effective dose of 0.3 mg/kg ($p \leq 0.025$). Plasma IRI levels appeared to be inversely proportional to the plasma glucose levels. At 10 min after the glucose load, plasma IRI levels were statistically increased ($p \leq 0.025$) at all doses except the 0.03 mg/kg, as shown in Fig. 2B and D. As with the plasma glucose, the increases in IRI levels were dose-dependent.

Effects of alogliptin on N-STZ-1.5 rats with SU secondary failure

A rat model of SU secondary failure was generated via the administration of glibenclamide at a dose of 10 mg/kg/day to N-STZ 1.5 rats for a total of 27 days. In addition, a second group of N-STZ-1.5 rats was administered vehicle for 27 days and was used as a control. In the rats administered vehicle for 27 days, a single dose of 10 mg/kg glibenclamide resulted in a significant improvement in glucose tolerance (Fig. 3A, B) with significant increase of plasma IRI levels at 0 min (before glucose load, when compared to the vehicle treated rats (0.43 ± 0.11 ng/ml vs. 1.20 ± 0.14 ng/ml, $p \leq 0.01$ by Student's *t*-test), as shown in Fig. 3C. However, the rats treated with glibenclamide at a dose of 10 mg/kg for 27 days exhibited exacerbation of glucose tolerance compared with the rats treated with vehicle for 27 days (Fig. 3). Even after a single dose of glibenclamide (10 mg/kg) or

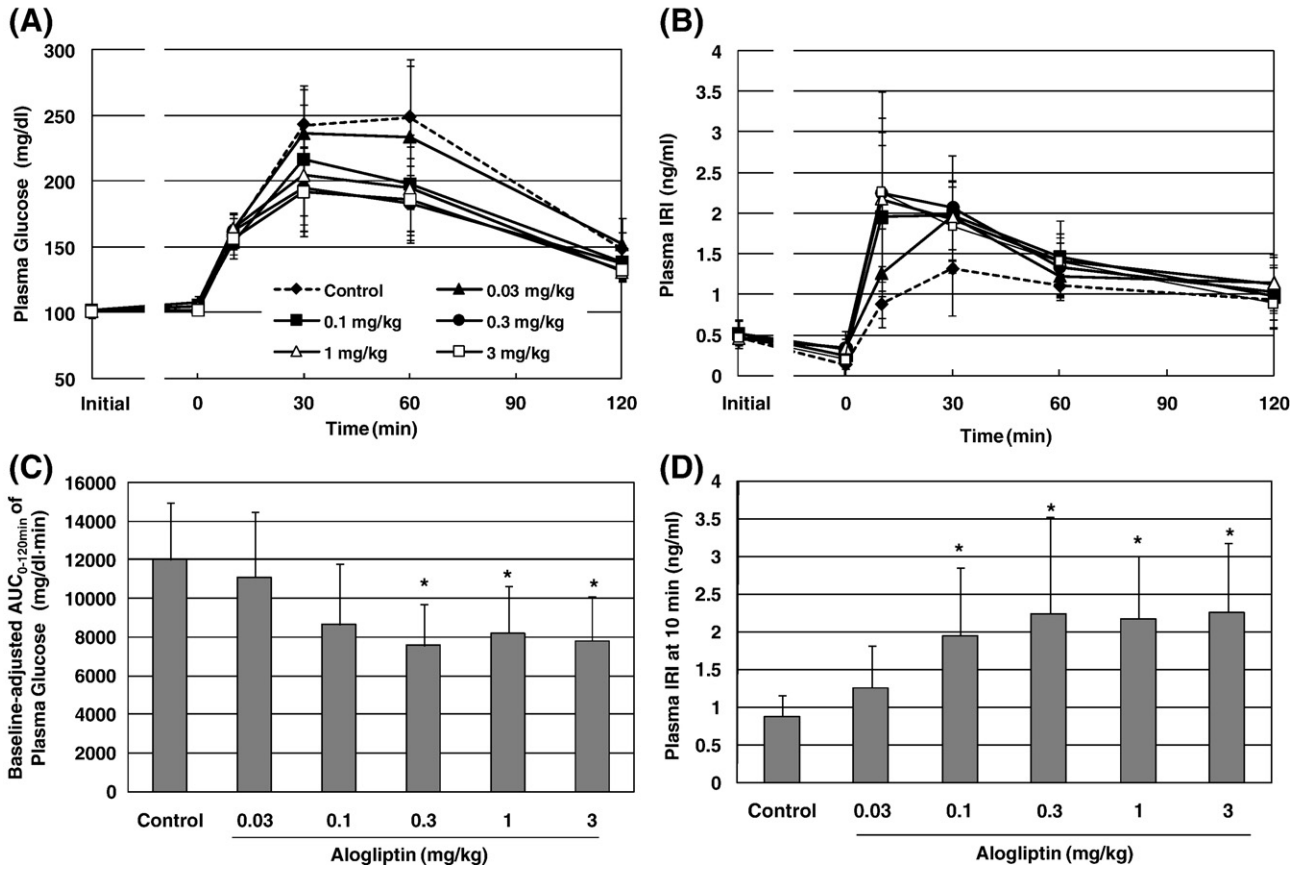

Fig. 2. Effects of alogliptin on plasma glucose (A), plasma IRI (B) baseline (0 min)-adjusted area under the concentration–time curve $(AUC)_{0-120\ min}$ of plasma glucose levels (C) and plasma IRI levels at 10 min after the oral glucose load (D) in an oral glucose tolerance test in N-STZ-1.5 rats. Values are mean and SD ($n = 6$). Alogliptin produced dose-dependent improvements in glucose tolerance with a minimum effective dose of 0.3 mg/kg. In addition, plasma IRI levels at 10 min after glucose load increased in a dose-dependent manner. *$p \leq 0.025$ compared with control by one-tailed Williams' test.

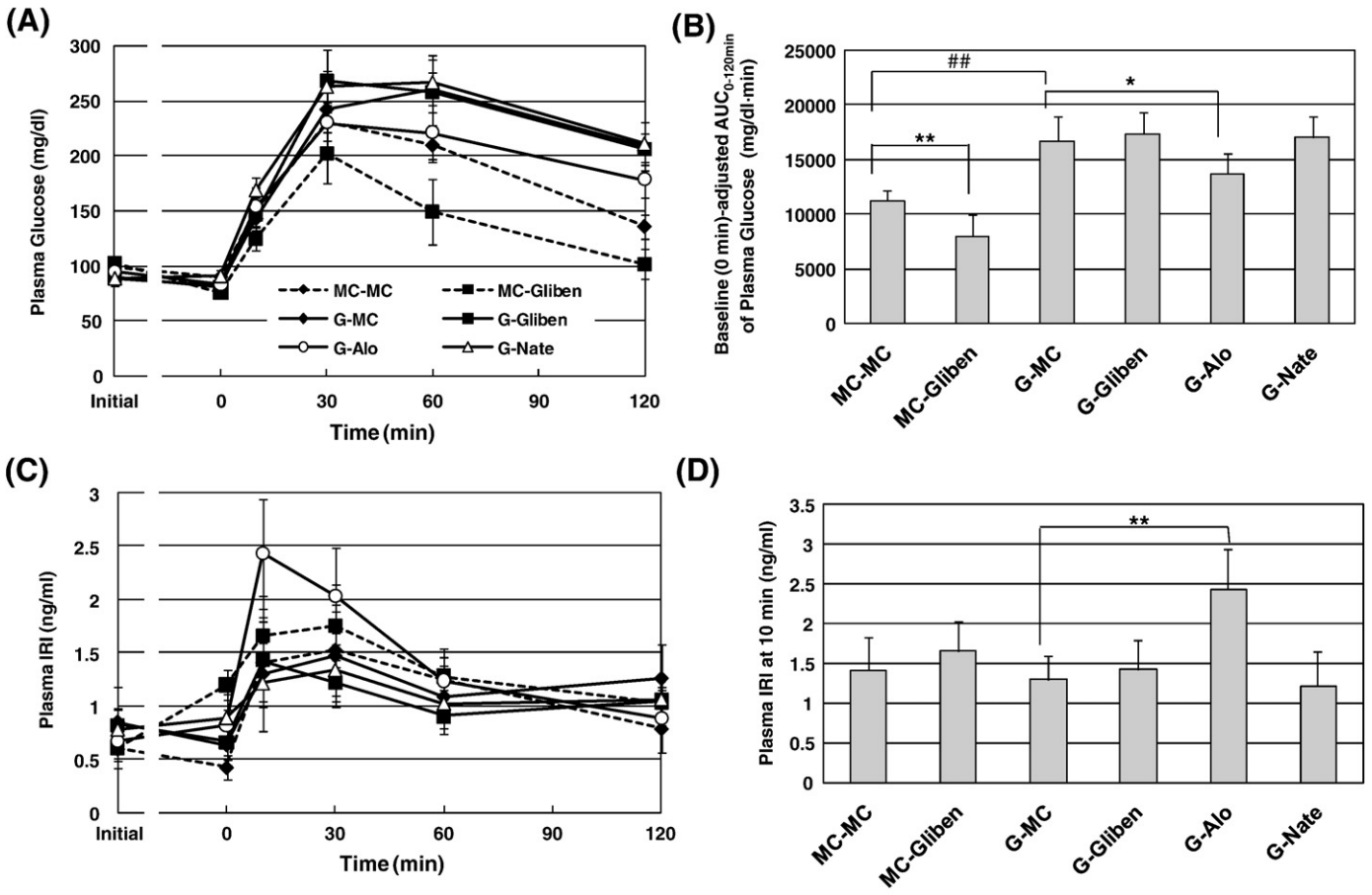

Fig. 3. Effects of alogliptin on plasma glucose (A), baseline (0 min) adjusted $AUC_{0-120\ min}$ of plasma glucose (B), plasma immuno-reactive insulin (IRI) (C) and IRI at 10 min after glucose load (D) in glucose tolerance test of N-STZ-1.5 rats. The animals were administered vehicle ($n=12$) or glibenclamide orally at a dose of 10 mg/kg ($n=24$) once daily for 27 days. After an overnight fast, the rats treated with vehicle for 27 days were given vehicle (MC-MC) or glibenclamide orally at a dose of 10 mg/kg (MC-Gliben) and the rats treated with glibenclamide for 27 days were given vehicle (G-MC), glibenclamide at a dose of 10 mg/kg (G-Gliben), alogliptin at a dose of 1 mg/kg (G-Alo) or nateglinide at a dose of 50 mg/kg (G-Nate). One hour (vehicle, glibenclamide and alogliptin) or 0.5 h (nateglinide) after dosing, all animals received an oral glucose load (1 g/kg). Values are mean and SD ($n=6$). *, **$p\leq0.05$ and $p\leq0.01$, respectively, by Student's t-test. ##$p\leq0.01$ by Aspin–Welch test.

nateglinide (50 mg/kg), a short acting insulinotropic that acts on SU receptors, plasma insulin levels before (0 min) and after oral glucose load could not be increased in these rats (Fig. 3C and D) and glucose tolerance was not improved (Fig. 3A and B). Interestingly, although SU secondary failure was evident, a single 1 mg/kg dose of alogliptin significantly improved ($p\leq0.05$) glucose excursion as evidenced by the decrease in plasma glucose levels (Fig. 3A and B) and exhibited a significant increase ($p\leq0.05$) of plasma IRI levels (Fig. 3C and D). Although it was not significant, consistent with the lower insulin secretory response with glibenclamide administration, pancreatic insulin content after 4-weeks of treatment with glibenclamide decreased 15% vs. vehicle (16.6 ± 2.9 ng/mg tissue vs. 14.1 ± 3.0 ng/mg tissue).

Discussion

Alogliptin is a novel, oral, potent, and highly selective DPP-4 inhibitor under development as a once-daily treatment for type 2 diabetes. Studies have shown that a single dose of alogliptin significantly improves oral glucose tolerance and early-phase insulin secretion in female Wistar fatty rats, a rat model that develops obesity and obesity-related features such as glucose intolerance, hyperinsulinemia and hyperlipemia (Feng et al. 2007). In the present study, N-STZ-1.5 rats were used to assess the antidiabetic effect of alogliptin in a non-obese diabetic model with impaired insulin secretion. Acute administration of alogliptin resulted in a significant decrease in plasma DPP-4 activity, and increased plasma active GLP-1. These data suggest that the increase in plasma active GLP-1 observed in the N-STZ-1.5 rats was a result of the inhibition of plasma DPP-4 activity. In addition, alogliptin improved glucose tolerance at a dose of 0.3 mg/kg and higher, with a dose-dependent increase in plasma IRI, suggesting that improved glucose tolerance results from the ability of alogliptin to enhance insulin secretion. Taken together, these data indicate that alogliptin improves glucose tolerance ultimately by preventing active GLP-1 degradation through the inhibition of DPP-4. Furthermore, these data suggest that alogliptin is effective in both obese rats, as shown previously (Feng et al. 2007) and non-obese diabetic rats as observed in this study.

The rate of SU secondary failure is higher than with other drugs such as thiazolidinediones and biguanide (Kahn et al. 2006) and was found to be 7% with gliclazide, 17.9% with glibenclamide and 25.6% with glipizide over 5 years (Harrower and Wong 1990). Many patients with SU secondary failure eventually convert to insulin treatment to better control blood glucose levels. There are several factors that are implicated in the development of SU secondary failure, including desensitization of the β-cells by long-term over-stimulation (Rustenbeck 2002), degranulation (Ling et al. 2006), variant of genes (Sesti et al. 2004, 2006), and progression of diabetes itself.

A rat model of SU secondary failure was developed using glibenclamide to estimate the effectiveness of alogliptin on the treatment of patients experiencing this phenomenon. N-STZ-1.5 rats were given glibenclamide or vehicle for 27 days after which an OGTT was performed on both groups of rats. A single dose of glibenclamide given to the rats treated with glibenclamide for 27 days was unable to increase plasma

insulin before and after the oral glucose load and did not improve glucose tolerance, in contrast to the vehicle treated rats. In addition, nateglinide, a short acting insulinotropic agent that acts on SU receptors, at a dose of 50 mg/kg failed to improve glucose tolerance. These results confirmed that glibenclamide-induced secondary failure was developed in the N-STZ-1.5 rats.

Pancreatic insulin content of our N-STZ-1.5 rats was decreased by about one half compared with normal rats (data not shown). Since the N-STZ-1.5 rat is basically a model of impaired insulin secretion, further decrease in insulin content could influence the glucose tolerance. Thus, although the decrease in pancreatic insulin content was not significant, degranulation of pancreatic β-cells could contribute to the progression of SU secondary failure in these animals.

Sulfonylureas act directly on pancreatic islet β-cells to close adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP} channels) in the cell membrane. Closure of K_{ATP} channels induces the elevation of intracellular calcium and promotes insulin secretion. Although nateglinide does not have a SU structure, it does stimulate insulin secretion through the closure of K_{ATP} channels. Therefore, it was not surprising that nateglinide was unable to enhance insulin release or lower the plasma glucose levels, similar to what was observed with glibenclamide. In contrast, oral administration of alogliptin, at a dose of 1 mg/kg, significantly improved glucose tolerance and increased insulin secretion in the N-STZ-1.5 rats exhibiting SU secondary failure. Furthermore, in contrast to glibenclamide, studies have shown that administration of alogliptin to N-STZ-1.5 rats for 4 weeks resulted in a slight dose-dependent increase in pancreatic insulin content (unpublished data). This suggests that alogliptin by itself has a low risk of secondary failure, which would be a tremendous benefit of this antidiabetic drug.

Conclusion

In conclusion, these results indicate that alogliptin demonstrated an antidiabetic effect in a non-obese type 2 diabetic rat model and was effective in diabetic rats exhibiting SU secondary failure, improving both glucose tolerance and insulin secretion. This suggests that alogliptin may be an effective alternative in treating type 2 diabetic patients exhibiting SU secondary failure.

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