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# Antidiabetic effects of dipeptidyl peptidase–IV inhibitors and sulfonylureas in streptozotocin-nicotinamide–induced mildly diabetic mice

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**Abstract**

The present study investigated the antidiabetic effects of the dipeptidyl peptidase (DPP)–IV inhibitors ASP8497 and vildagliptin, and the sulfonylureas glibenclamide and gliclazide in streptozotocin-nicotinamide–induced mildly diabetic mice. A single administration of ASP8497 and vildagliptin significantly improved glucose tolerance by increasing plasma insulin and glucagon-like peptide–1 levels. In addition, a single administration of glibenclamide and gliclazide also caused significant improvement in glucose tolerance with an accompanying increase in the plasma insulin level. Subsequently, the effects of a 1-week chronic daily dosing of DPP-IV inhibitors and sulfonylureas were investigated. All drugs significantly improved glucose tolerance on day 1 of chronic daily dosing. After 1 week of chronic daily dosing, the DPP-IV inhibitors caused a significant improvement in glucose tolerance similar to those observed on day 1 by increasing the plasma insulin and glucagon-like peptide–1 levels. In contrast, the sulfonylureas had no significant improving or insulinotropic effect. Furthermore, ASP8497 also had an antihyperglycemic effect and improved pancreatic histopathologic lesions in a 4-week chronic daily dosing study. These results suggest that chronic daily dosing of sulfonylureas had virtually no antidiabetic effects because of marked attenuation of the insulinotropic action in streptozotocin-nicotinamide–induced mildly diabetic mice. In contrast, the antidiabetic efficacy of DPP-IV inhibitors, including ASP8497, did not change even after chronic daily dosing; therefore, DPP-IV inhibitors are useful as a therapeutic agent for impaired glucose tolerance and type 2 diabetes mellitus.


## 1. Introduction

Type 2 diabetes mellitus, affecting more than 90% of diabetic patients, is a heterogenous and polygenic metabolic disease characterized by hyperglycemia and insulin resistance. The pathologic mechanisms of this disease are mainly attributed to impaired insulin secretion by pancreatic $\beta$-cells and insulin resistance in target tissues, including skeletal muscle and the liver, which leads to hyperglycemia [1]. Of the oral antidiabetic agents currently available, sulfonylureas have a superior postprandial antihyperglycemic effect because of their potent insulinotropic action and are effective in many diabetic patients, with few primary nonresponders [2-4]. However, findings in both basic studies and clinical practice indicate that sulfonylureas cause hypoglycemic symptoms associated with persistent insulin secretion irrespective of blood glucose levels and efficacy attenuation in long-term treatment, that is, secondary failure, as a common undesirable adverse effect [5-8]. Suggested causes of this secondary failure include (1) a decrease in the pancreatic insulin content associated with potent and sustained insulin secretion, (2) desensitization resulting from hyperstimulation of pancreatic $\beta$-cells, and (3) responsiveness of $\beta$-cells to glucose toxicity under hyperglycemic conditions [9-12]; but precise mechanisms remain unclear.

In recent years, glucagon-like peptide (GLP)–1 has been actively investigated for use as a novel therapeutic agent in the treatment of type 2 diabetes mellitus [13]. Glucagon-like peptide–1 is a gastrointestinal hormone secreted into circulation from the L cells of the small intestine and

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proximal colon in response to the ingestion of nutrients, and increases glucose-dependent insulin secretion through the GLP-1 receptors expressed on pancreatic β-cells [14]. However, GLP-1 action has a very short life of about 1 minute because of its degradation by dipeptidyl peptidase (DPP)–IV (EC 3.4.14.5, CD26) [15]. Dipeptidyl peptidase–IV is a serine protease that removes the dipeptides from the N-terminus of substrate peptides by cleaving postproline or alanine residues. Dipeptidyl peptidase–IV is expressed in body fluids and many tissues, including the kidney and liver, and exists as either a soluble or a membrane-bound enzyme [16]. It has been demonstrated that this residue cleavage is the primary physiologic route of GLP-1 degradation in both humans and animals [17,18]. Since the discovery that postprandial GLP-1 secretion was impaired, but retained its potency in type 2 diabetes mellitus [19,20], several approaches have been used to enhance GLP-1 action, thereby ameliorating type 2 diabetes mellitus. Of these, the inhibition of DPP-IV prevents proteolytic degradation of biologically active GLP-1 and enhances glucose-dependent insulin secretion from pancreatic β-cells. In animal studies, this led to a reduction in postprandial hyperglycemia without affecting fasting blood glucose levels [21,22]. Furthermore, clinical efficacy has been reported for DPP-IV inhibitors such as sitagliptin and vildagliptin [23,24]. Previous reports have shown that DPP-IV inhibitors have antihyperglycemic effects in diabetic animals and patients; but very few reports have shown whether efficacy attenuation occurs because of chronic daily dosing, as it does for sulfonylureas. Therefore, the present study compared antidiabetic effects after single and chronic daily dosing of the DPP-IV inhibitors ASP8497 [25] and vildagliptin [26] to the sulfonylureas glibenclamide and gliclazide in streptozotocin-nicotinamide–induced mildly diabetic mice.

## 2. Materials and methods

### 2.1. Compounds

ASP8497 and vildagliptin were synthesized by Astellas Pharma (Ibaraki, Japan). Glibenclamide and gliclazide were purchased from Sigma-Aldrich (St Louis, MO). These compounds were dissolved or suspended in 0.5% methylcellulose solution and then orally administered.

### 2.2. Animals

Male ICR mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and used at the age of 6 weeks. Streptozotocin-nicotinamide–induced diabetic mice were created by treating the mice as follows: fasting overnight and intraperitoneally administering a nicotinamide solution (1000 mg/kg), followed by a streptozotocin solution (150 mg/kg) 90 minutes later. Normal control mice were intraperitoneally administered physiologic saline. Blood glucose levels were measured in the diabetic mice 1 week later, after which they were grouped so that the blood glucose levels were uniform among the groups. All mice were housed under conventional conditions with controlled temperature, humidity, and light (12-hour light-dark cycle), and were provided with a standard commercial diet (CE-2; Oriental Yeast, Tokyo, Japan) and water (ad libitum). All experimental procedures were conducted according to the Animal Ethical Committee of Astellas Pharma.

### 2.3. Single-administration study

For examination of the blood glucose level, blood samples were collected from normal and diabetic mice fasted overnight and to which either the vehicle or the test compound (ASP8497 and vildagliptin, 0.1-3 mg/kg; glibenclamide and gliclazide, 3-30 mg/kg) had been orally administered. After 30 minutes, the blood glucose levels were measured, followed by the oral administration of glucose solution (2 g/kg). The blood glucose levels were measured 0.5, 1, and 2 hours after glucose loading. For the examination of plasma insulin and GLP-1 levels, normal and diabetic mice that had been fasted overnight were orally administered either the vehicle or test compound (ASP8497 and vildagliptin, 3 mg/kg; glibenclamide and gliclazide, 10 mg/kg), followed 30 minutes later by the oral administration of glucose solution. Blood samples were collected 10 minutes after glucose loading.

### 2.4. One-week chronic daily dosing study

The effects of the test compounds (ASP8497 and vildagliptin, 3 mg/kg; glibenclamide and gliclazide, 10 mg/kg) on the blood glucose level were examined on day 1 of chronic daily dosing, as in the single-administration study described above. Subsequently, oral administration of vehicle or test compound was continued at the same dose once daily; and the effects of the test compound on the blood glucose level were examined again on day 7. To examine plasma insulin and GLP-1 levels and pancreatic insulin content, drug administration was continued at the same dose. On day 9 of chronic daily dosing, glucose solution was orally administered 30 minutes after the test compound dosing. Blood samples were collected 10 minutes later, after which the pancreas was isolated under ether anesthesia.

### 2.5. Four-week chronic daily dosing study

From the age of 6 weeks, mice were fed only during the dark (active) period (8:00 PM to 8:00 AM); this controlled feeding continued during the study period. One week later, diabetes was induced by giving streptozotocin and nicotinamide, as described above. After an additional week, the animals were assigned to receive vehicle or ASP8497 (5 mg/kg) immediately before feeding, once daily for 4 weeks. On the morning after final administration, blood samples were collected under nonfasting conditions; and the pancreas was isolated. Part of the isolated pancreas was cryopreserved for measurement of insulin content, and the remaining part was immersed and fixed in phosphate-

buffered 10% formalin solution to prepare a paraffin section. This section was histopathologically evaluated after staining with hematoxylin and eosin and immunostaining with an anti-mouse insulin antibody (Insulin Ab-6 [INS04 + INS05]; Lab Vision, Fremont, CA).

### 2.6. Biochemical determination

Blood glucose levels were measured using Glucose CII-Test reagent (Wako Pure Chemical Industries, Osaka, Japan). Cryopreserved pancreas was homogenized by adding acid-ethanol solution (75% ethanol, 23.5% purified water, and 1.5% concentrated hydrochloric acid) and incubating at 4°C for 1 hour to extract the insulin. Subsequently, the culture was centrifuged; and the supernatant was used as a measurement sample. Plasma and pancreatic insulin concentrations were measured using a Biotrak rat insulin radioimmunoassay kit (Amersham Biosciences, Piscataway, NJ). Plasma GLP-1 concentrations were measured using an active GLP-1 enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO). Hemoglobin $A_{1c}$ ($HbA_{1c}$) levels were measured using a DCA2000 System (Bayer Medical, Tokyo, Japan).

### 2.7. Statistical analysis

The experimental results are expressed as the mean ± SE. The significance of differences between normal and diabetic animals receiving the vehicle was determined using the Student *t* test. The significance of differences between the vehicle group and test compound groups was assessed using the Dunnett multiple range test. A value of *P* less than .05 was taken to be significant. Statistical and data analyses were conducted using the SAS 8.2 software package (SAS Institute, Tokyo, Japan).

## 3. Results

### 3.1. Single-administration study

In the oral glucose tolerance test, streptozotocin-nicotinamide–induced mildly diabetic mice exhibited significant aggravation of glucose tolerance because of the loss of early-phase insulin secretion. ASP8497 (0.1-3 mg/kg) and vildagliptin (0.1-3 mg/kg) caused a significant and dose-dependent improvement in glucose tolerance, with significant increases in plasma insulin and GLP-1 levels (Fig. 1). Glibenclamide (3-30 mg/kg) and gliclazide (3-30 mg/kg) also caused a dose-dependent and significant improvement in glucose tolerance, with a significant increase in the plasma insulin level; however, no change in plasma GLP-1 level was observed (Fig. 2).

### 3.2. One-week chronic daily dosing study

On the first day of the 1-week chronic daily dosing study, ASP8497 (3 mg/kg), vildagliptin (3 mg/kg), glibenclamide

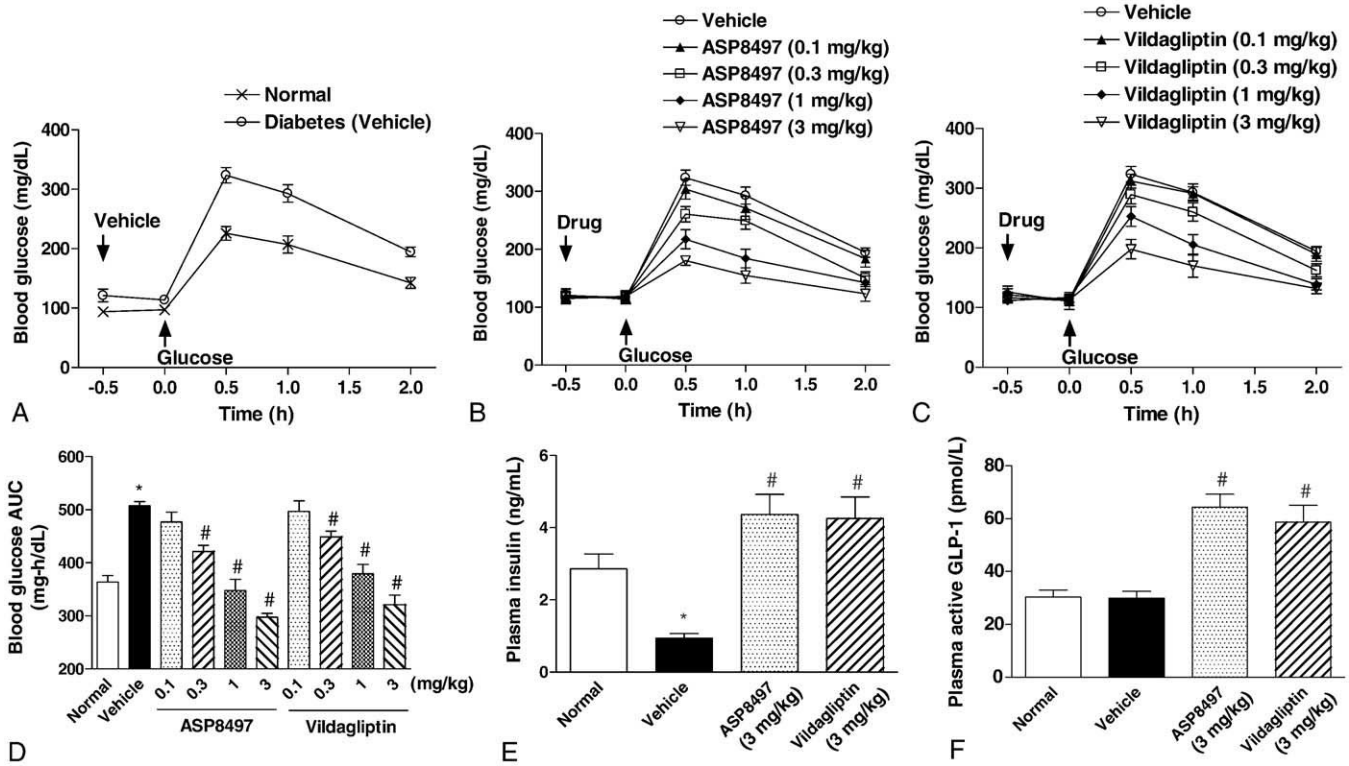


Fig. 1. Effects of ASP8497 and vildagliptin on blood glucose, plasma insulin, and GLP-1 levels during the oral glucose tolerance test (OGTT) in diabetic mice. A-C, Time course of changes in blood glucose levels and (D) the area under the blood glucose concentration-time curve (AUC) during the OGTT. Plasma (E) insulin and (F) active GLP-1 levels at minute 10 during the OGTT. The values represent the mean ± SE for 5 animals in each group. *$P < .05$ vs normal group; #$P < .05$ vs vehicle group.

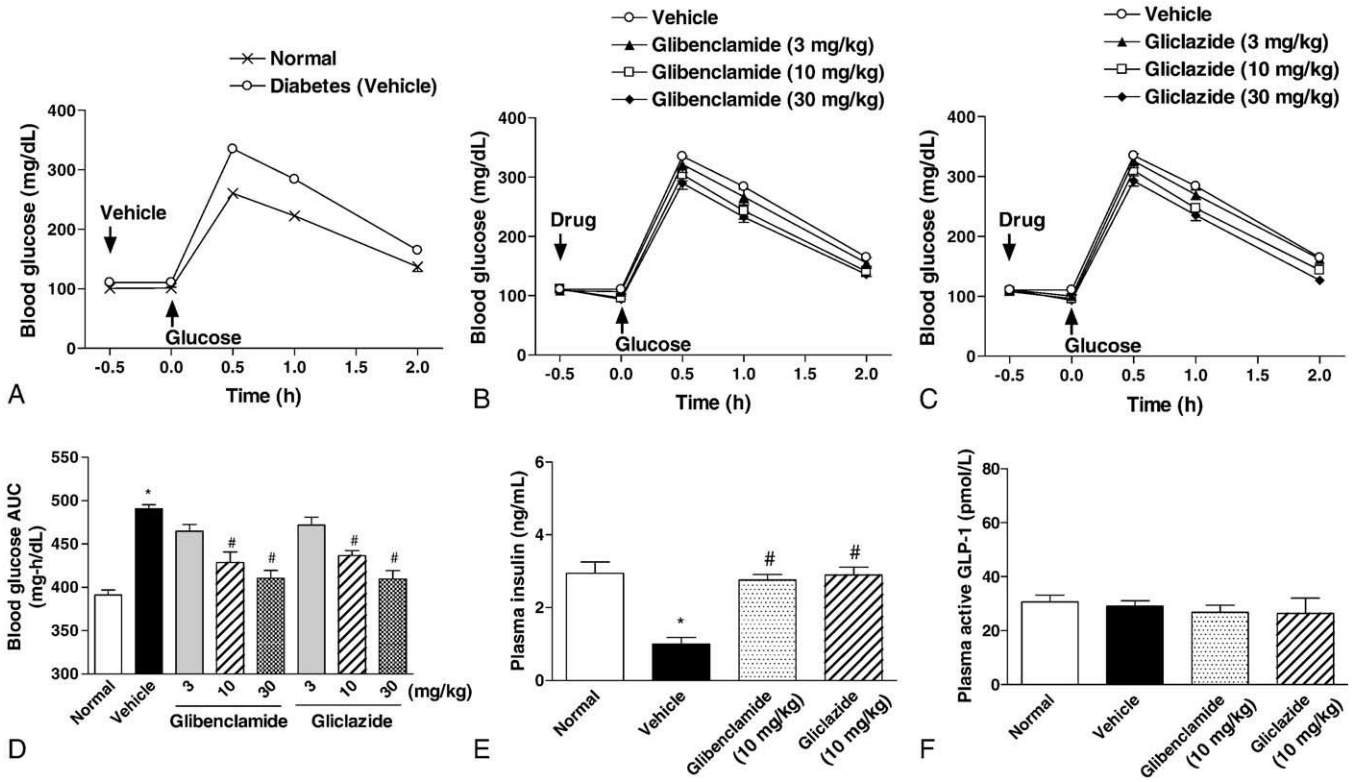


Fig. 2. Effects of glibenclamide and gliclazide on blood glucose, plasma insulin, and GLP-1 levels during the OGTT in diabetic mice. A-C, Time course of changes in blood glucose levels and (D) the AUC during the OGTT. Plasma (E) insulin and (F) active GLP-1 levels at minute 10 during the OGTT. The values represent the mean ± SE for 5 animals in each group. *$P < .05$ vs normal group; #$P < .05$ vs vehicle group.

(10 mg/kg), and gliclazide (10 mg/kg) all caused a significant improvement in glucose tolerance (Fig. 3A-C). On day 7 of chronic daily dosing, ASP8497 and vildagliptin caused a significant improvement in glucose tolerance, as observed on day 1; however, glibenclamide and gliclazide caused no significant improving effects (Fig. 3D-F). On day 9 of chronic daily dosing, significant increases in the plasma GLP-1 and insulin levels were obtained after administration of ASP8497 and vildagliptin; but no increase in the plasma insulin level was observed after administration of glibenclamide or gliclazide (Fig. 4A and B). Furthermore, ASP8497 and vildagliptin significantly increased pancreatic insulin content; but glibenclamide and gliclazide caused no significant changes (Fig. 4C).

### 3.3. Four-week chronic daily dosing study

In the 4-week chronic daily dosing study, ASP8497 (5 mg/kg) significantly decreased nonfasting blood glucose and $HbA_{1c}$ levels (Table 1). Histopathologic evaluation of the pancreata of diabetic mice revealed a high frequency of degenerative changes, such as a moderate decrease in the number of insulin-positive granules as well as atrophy, pyknosis, degeneration, and necrosis in the islets (Fig. 5, Table 2). In contrast, degenerative changes occurred at a low frequency in the ASP8497 group, with only a slight decrease in the number of insulin-positive granules and no marked islet atrophy, degeneration, or necrosis.

## 4. Discussion

Primary defects in insulin secretion, along with the development of insulin resistance, contribute to the etiology of type 2 diabetes mellitus. Diminished postprandial insulin secretion resulting from both functional defects and the loss of pancreatic $\beta$-cells progresses to hyperglycemia and declining insulin sensitivity [27]. Therefore, sulfonylureas, antihyperglycemic drugs with potent insulinotropic effects, are widely used for patients with type 2 diabetes mellitus. However, hypoglycemia is a known adverse effect of these drugs due to their glucose-independent and long-lasting induction of insulin secretion [6]. In contrast, DPP-IV inhibitors, which have a glucose-dependent insulinotropic effect, can be used as safe drugs because of their low risk of hypoglycemia induction. Previous studies have shown that ASP8497 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 in streptozotocin-nicotinamide–induced mildly diabetic mice and Zucker fatty rats [25,28]. In addition, sulfonylureas significantly decreased

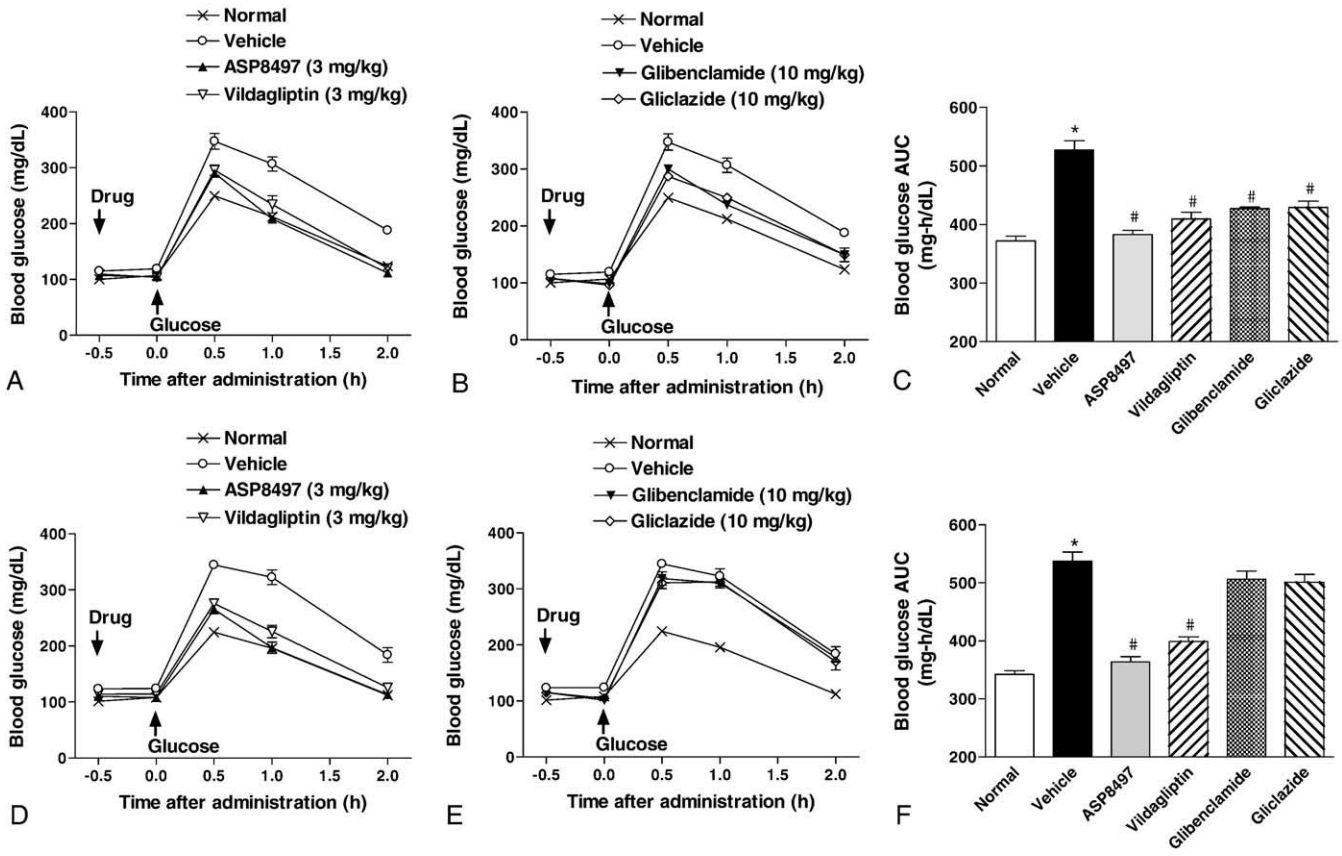


Fig. 3. Effects of chronic daily dosing of DPP-IV inhibitors and sulfonylureas on glucose tolerance in diabetic mice. Time course of changes in blood glucose levels and the AUC during the OGTT on the (A-C) first and (D-F) seventh day of chronic daily dosing. The values represent the mean ± SE for 5 animals in each group. *$P < .05$ vs normal group; #$P < .05$ vs vehicle group.

the blood glucose level in fasted normal mice; but ASP8497 did not. Therefore, the hypoglycemic risk of ASP8497 is low. Furthermore, 13-week repeated oral administration study in rats was conducted that confirmed the safety of doses up to 100 mg/kg (unpublished data). The present study demonstrated that the DPP-IV inhibitors ASP8497 and vildagliptin have a glucose tolerance–improving effect comparable with or superior to that of the sulfonylureas glibenclamide and gliclazide, which suggests their usefulness as a therapeutic agent for type 2 diabetes mellitus.

In this study, ASP8497 (3 mg/kg) significantly increased the plasma GLP-1 level (vehicle, 30 pmol/L; ASP8497, 64 pmol/L). In the previous study using the same diabetic model, the same dose of ASP8497 also significantly increased the plasma GLP-1 level; however, this result was different from that in the present study (vehicle, 4 pmol/L; ASP8497, 32 pmol/L). During the preliminary examination, the highest plasma GLP-1 level during the oral glucose tolerance test was found to occur 5 to 10 minutes after glucose loading; therefore, the plasma GLP-1 level was measured 10 minutes after glucose loading in the efficacy evaluation studies. Because changes (increase and reduction) in the plasma GLP-1 level accompanying the secretion of GLP-1 caused by glucose loading as well as decomposition by DPP-IV are very fast, the plasma GLP-1 level during the oral glucose tolerance test was presumed to differ between studies, especially in the absence of DPP-IV inhibitor. The fact that slight differences in the disease state of the diabetic mice in different examinations could also affect the results cannot be denied either. However, in both examinations, ASP8497 caused not only a significant increase in the plasma GLP-1 level, but also a significant increase in plasma insulin level and improvement of glucose tolerance; thus, these differences are thought to have no substantial effect on the evaluation of the study results.

Although sulfonylureas show antihyperglycemic effects associated with insulin secretion in patients with type 2 diabetes mellitus, there is still the problem of attenuation of insulinotropic action that occurs in some patients undergoing long-term treatment. The UK Prospective Diabetes Study has also reported that as many as 48% of patients using glibenclamide and 40% of patients using chlorpropamide changed to or added another drug because of aggravation of their condition during a 6-year treatment period [8]. The present study also showed that, when streptozotocin-nicotinamide–induced mildly diabetic mice received chronic daily dosing of glibenclamide and

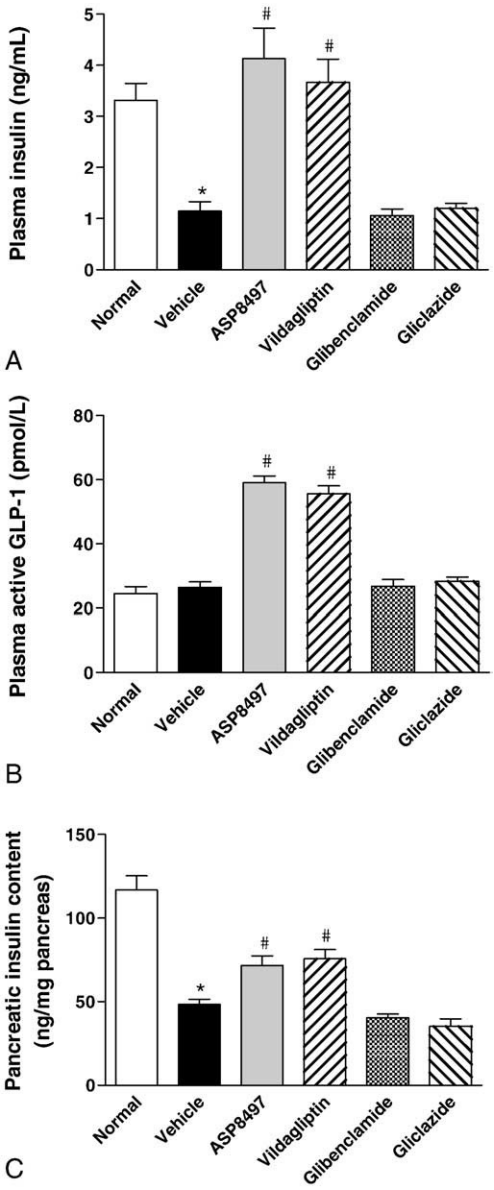


Fig. 4. Effects of chronic daily dosing of DPP-IV inhibitors and sulfonylureas in diabetic mice. Plasma (A) insulin and (B) active GLP-1 levels at minute 10 during the OGTT on the ninth day of chronic daily dosing. C, Pancreatic insulin content after the OGTT. The values represent the mean ± SE for 5 animals in each group. *$P < .05$ vs normal group; #$P < .05$ vs vehicle group.

gliclazide for 1 week, both sulfonylureas significantly improved glucose tolerance on the first day, but their insulinotropic action was markedly attenuated on day 7, with no glucose tolerance–improving effect. Hosokawa and Leahy [9] and Gold et al [29] explained that the efficacy attenuation seen with chronic daily dosing of sulfonylureas was due to decreased pancreatic function resulting from the depletion of insulin content associated with potent and long-lasting insulin secretion. In addition, Kawaki et al [12] suggested that chronic treatment with sulfonylureas causes a defect in insulin secretion by reducing the number of functional adenosine triphosphate–sensitive $K^+$ channels on the plasma membrane of the $\beta$-cells. Furthermore, Sako and Grill [30] suggested the possibility of a decreased $\beta$-cell response due to glucose toxicity under hyperglycemic conditions. Currently, however, the details of the mechanism behind efficacy attenuation by sulfonylureas remain unknown. In the present study, pancreatic insulin content was measured on day 9 of chronic daily dosing when efficacy of sulfonylureas had almost disappeared. Both sulfonylureas glibenclamide and gliclazide showed no significant difference from the vehicle group, with no depletion of pancreatic insulin content. Therefore, these findings suggest the possibility that the efficacy attenuation resulting from chronic daily dosing of sulfonylureas in streptozotocin-nicotinamide–induced mildly diabetic mice is not caused by depletion of pancreatic insulin content, but rather by an abnormal mechanism that involves desensitization relating to insulin secretion in the pancreas. In contrast, both of the DPP-IV inhibitors ASP8497 and vildagliptin caused a significant improvement in glucose tolerance on both days 1 and 7 of chronic daily dosing, with hardly any change in their efficacy. Furthermore, pancreatic insulin content on day 9 of chronic daily dosing caused significant increases compared with the vehicle group. These findings suggest that the DPP-IV inhibitor–induced effective glucose-dependent insulin secretion and accompanying blood glucose–lowering effects may lead to economization of insulin and increases in pancreatic insulin content.

In the present study, the antidiabetic effect of ASP8497 during 4 weeks of chronic daily dosing was examined further. To determine the dose of ASP8497 required for clinical efficacy, feeding was limited to during the active period only, to mimic human food intake patterns, and ASP8497 was orally administered immediately before feeding. Preliminary examination confirmed that, even when feeding is limited to only during the active period, diabetic mice show no marked differences in daily food intake; and the hyperglycemia associated with loss of early-phase insulin secretion is observed throughout the study period. ASP8497 produced significant decreases in nonfasting blood glucose and $HbA_{1c}$ levels. Furthermore, a histopathologic evaluation of the pancreas revealed that ASP8497 improved degenerative changes, including decreases in the number of insulin-positive granules as

Table 1
Antihyperglycemic effect of chronic daily dosing of ASP8497 in diabetic mice

| | Normal | Vehicle | ASP8497 |
|---|---|---|---|
| Nonfasting blood glucose (mg/dL) | 143 ± 7 | 233 ± 6* | 172 ± 7† |
| $HbA_{1c}$ (%) | 2.79 ± 0.03 | 3.57 ± 0.04* | 2.80 ± 0.11† |

The values represent the mean ± SE for 7 animals in each group.
* $P$ <05 vs normal group.
† $P < .05$ vs vehicle group.

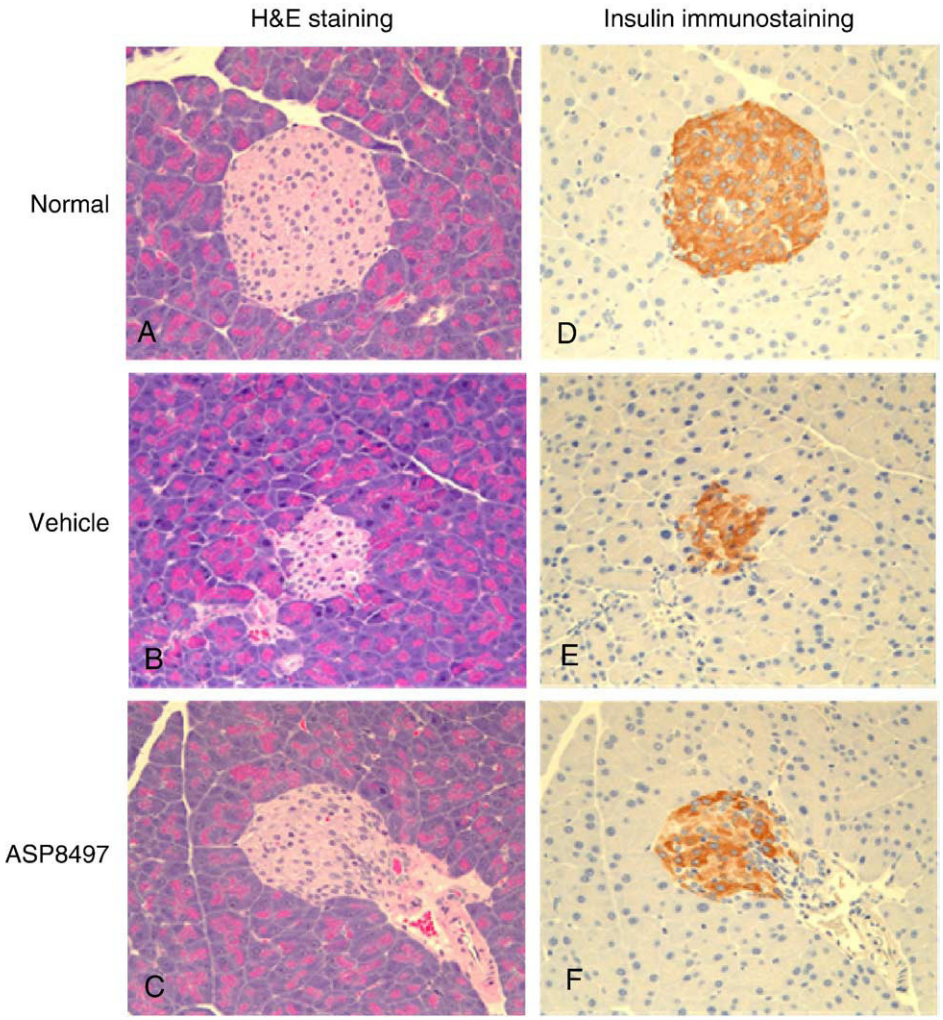


Fig. 5. Effect of chronic daily dosing of ASP8497 on histopathologic changes in the pancreata of diabetic mice. Hematoxylin and eosin staining and anti-insulin antibody immunostaining from (A, D) normal mice and from (B, E) vehicle-treated and (C, F) ASP8497 (5 mg/kg)–treated diabetic mice. Original magnification ×100. H&E indicates hematoxylin and eosin.

well as islet atrophy, degeneration, and necrosis. It has been confirmed that GLP-1 has not only glucose-dependent insulinotropic effects, but many other physiologic effects in pancreatic $\beta$-cells. In addition, its promotion of insulin biosynthesis, cell proliferation, and differentiation, and an apoptosis inhibitory action have also been demonstrated [31-33]. Therefore, the increases in pancreatic insulin content and improvement of pancreatic lesions seen after treatment with ASP8497 could be attributable at least in part to direct action on pancreatic $\beta$-cells mediated by GLP-1; ASP8497 may have not only a glucose tolerance–improving effect, but also a protective effect on the pancreas.

Table 2
Effects of chronic daily dosing of ASP8497 on pancreatic lesions in diabetic mice

| Histopathologic findings | Normal | Vehicle | ASP8497 |
|---|---|---|---|
| No. of animals | 7 | 7 | 7 |
| Islet atrophy | 0 | 7 | 0 |
| Pyknosis | 0 | 7 | 7 |
| Degeneration/necrosis | 0 | 7 | 0 |
| Insulin-positive granules | | | |
| Slight decrease | 0 | 2 | 6 |
| Moderate decrease | 0 | 5 | 1 |

In conclusion, the present results suggest that the DPP-IV inhibitor ASP8497 has a superior antidiabetic effect, including improvement in glucose tolerance in streptozotocin-nicotinamide–induced mildly diabetic mice. The drug has demonstrated the ability to control blood glucose levels with chronic daily dosing and holds promise as a useful therapeutic agent for type 2 diabetes mellitus.

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## References


[1] Cavaghan MK, Ehrmann DA, Polonsky KS. Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. J Clin Invest 2000;106:329-33.

[2] Groop LC. Sulfonylureas in NIDDM. Diabetes Care 1992;15:737-54.

[3] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. Lancet 1998;352:837-53.

[4] Holman RR. Long-term efficacy of sulfonylureas: a United Kingdom Prospective Diabetes Study perspective. Metabolism 2006;55:S2-5.

[5] Asplund K, Wiholm BE, Lithner F. Glibenclamide-associated hypoglycaemia: a report on 57 cases. Diabetologia 1983;24:412-7.

[6] Stahl M, Berger W. Higher incidence of severe hypoglycaemia leading to hospital admission in type 2 diabetic patients treated with long-acting versus short-acting sulphonylureas. Diabet Med 1999;16: 586-90.

[7] Pontiroli AE, Calderara A, Pozza G. Secondary failure of oral hypoglycaemic agents: frequency, possible causes, and management. Diabetes Metab Rev 1994;10:31-43.

[8] Matthews DR, Cull CA, Stratton IM, Holman RR, Turner RC. UKPDS 26: sulphonylurea failure in non–insulin-dependent diabetic patients over six years. Diabet Med 1998;15:297-303.

[9] Hosokawa YA, Leahy JL. Parallel reduction of pancreas insulin content and insulin secretion in 48-h tolbutamide-infused normoglycemic rats. Diabetes 1997;46:808-13.

[10] Leahy JL. Impaired $\beta$-cell function with chronic hyperglycemia: 'overworked $\beta$-cell' hypothesis. Diabetes Rev 1996;4:298-319.

[11] Taverna MJ, Pacher N, Bruzzo F, Slama G, Selam JL. Beta-cell function evaluated by HOMA as a predictor of secondary sulphonylurea failure in type 2 diabetes. Diabet Med 2001;18:584-8.

[12] Kawaki J, Nagashima N, Tanaka J, Miki T, Miyazaki M, Gonoi T, et al. Unresponsiveness to glibenclamide during chronic treatment induced by reduction of ATP-sensitive $K^+$ channel activity. Diabetes 1999;48:2001-6.

[13] Gromada J, Brock B, Schmitz O, Rorsman P. Glucagon-like peptide–1: regulation of insulin secretion and therapeutic potential. Basic Clin Pharmacol Toxicol 2004;95:252-62.

[14] Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev 2007;87:1409-39.

[15] MacDonald PE, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. Diabetes 2002;51:S434-42.

[16] Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul Pept 1999;85:9-24.

[17] Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 1995;136:3585-96.

[18] Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide 1 are rapidly degraded from the $NH_2$-terminus in type II diabetic patients and in healthy subjects. Diabetes 1995;44: 1126-31.

[19] Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. N Engl J Med 1992;326:1316-22.

[20] Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Highes TE, Michelsen BK, et al. Determinations of the impaired secretion of glucagon-like peptide–1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001;86:3717-23.

[21] Pederson RA, White HA, Schlenzig D, Pauly RP, McIntosh CH, Demuth HU. Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. Diabetes 1998;47:1253-8.

[22] Reimer MK, Holst JJ, Ahren B. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. Eur J Endocrinol 2002;146:717-27.

[23] Raz I, Hanefeld M, Xu L, Caria C, Williams-Herman D, Khatami H, et al. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. Diabetologia 2006;49:2564-71.

[24] Rosenstock J, Baron MA, Dejager S, Mills D, Schweizer A. Comparison of vildagliptin and rosiglitazone monotherapy in patients with type 2 diabetes: a 24-week, double-blind, randomized trial. Diabetes Care 2007;30:217-23.

[25] Someya Y, Tahara A, Nakano R, Matsuyama-Yokono A, Nagase I, Fukunaga Y, et al. Pharmacological profile of ASP8497, a novel, selective, and competitive dipeptidyl peptidase–IV inhibitor, in vitro and in vivo. Naunyn Schmiedebergs Arch Pharmacol 2008;377: 209-17.

[26] Villhauer EB, Brinkman JA, Naderi GB, Burkey BF, Dunning BE, Prasad K, et al. 1-[[(3-Hydroxy-1-adamantyl]amino)acetyl]-2-cyano-(*S*)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. J Med Chem 2003;46:2774-89.

[27] Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, et al. Abnormal patterns of insulin secretion in non–insulin-dependent diabetes mellitus. N Engl J Med 1988;318:1231-9.

[28] Matsuyama-Yokono A, Tahara A, Nakano R, Someya Y, Nagase I, Hayakawa M, et al. ASP8497 is a novel selective and competitive dipeptidyl peptidase-IV inhibitor with antihyperglycemic activity. Biochem Pharmacol 2008;76:98-107.

[29] Gold G, Pou J, Gishizky ML, Landahl HD, Grodsky GM. Effects of tolbutamide pretreatment on the rate of conversion of newly synthesized proinsulin to insulin and the compartmental characteristics of insulin storage in isolated rat islets. Diabetes 1986;35:6-12.

[30] Sako Y, Grill VE. Coupling of beta-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. Diabetes 1990;39:1580-3.

[31] Brubaker PL, Drucker DJ. Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology 2004;145:2653-9.

[32] List JF, Habener JF. Glucagon-like peptide 1 agonists and the development and growth of pancreatic beta-cells. Am J Physiol 2004;286:E875-81.

[33] Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide–1 receptor signaling modulates beta cell apoptosis. J Biol Chem 2003;278:471-8.