Original Article

Acute and chronic administration of SHR117887, a novel and specific dipeptidyl peptidase-4 inhibitor, improves metabolic control in diabetic rodent models

Xiao LIU¹, Li-na ZHANG¹, Ying FENG¹, Lei ZHANG², Hui QU¹, Guo-qing CAO², Ying LENG¹ *

¹State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; ²Shanghai Hengrui Pharmaceuticals Co, Ltd, Shanghai 201203, China

Aim: Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of anti-diabetic agents. The purpose of this study was to assess the acute and chronic effects of SHR117887, a novel DPP-4 inhibitor, on metabolic control and pancreatic β -cell function in normal or diabetic rodent models.

Methods: In the acute experiments, ICR mice, diet-induced obese (DIO) rats and *ob/ob* mice were subjected to an oral glucose tolerance test (OGTT) following a single oral administration of SHR117887 (0.1, 0.3, 1, or 3 mg/kg). Blood samples were collected to measure glucose, insulin, DPP-4 activity and active GLP-1 level. In the chronic experiments, *ob/ob* mice was administered SHR117887 (3, 10, or 30 mg/kg) twice daily for 33 d to assess the effects on metabolic control and pancreatic β -cell function. Vildagliptin (LAF237) was used as a positive control in all the experiments.

Results: Acute oral administration of SHR117887 dose-dependently decreased the serum DPP-4 activity and improved glucose tolerance in ICR mice, DIO rats and *ob/ob* mice. This was accompanied by significant increases in the serum active GLP-1 and insulin levels. Chronic administration of SHR117887 significantly decreased fasting blood glucose level and improved the lipid profiles in *ob/ob* mice by reducing the serum triglyceride and free fatty acid levels, and its efficacy was comparable with that of vildagliptin at the same molarity. Moreover, chronic administration of SHR117887 increased the insulin staining of islet cells, which is suggestive of improved β -cell function.

Conclusion: SHR117887 is a potent DPP-4 inhibitor that improves metabolic control and β -cell function in diabetic rodent models, suggesting that it could be a new therapeutic agent for the treatment of type 2 diabetes.

Keywords: SHR117887; vildagliptin (LAF237); dipeptidyl peptidase-4 (DPP-4); type 2 diabetes mellitus; glucagon-like peptide-1 (GLP-1); insulin; β -cells

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Introduction

Type 2 diabetes mellitus (T2DM) is considered to be a chronic metabolic disease due to insulin resistance and pancreatic β -cell dysfunction. It is characterized by impaired insulin responsiveness and anatomical abnormalities of the pancreatic islets during the course of the disease development^[1, 2]. Due to the increasing prevalence of T2DM, the high burden of its complications, the limitation of adequate long-term glycemic control and the adverse effects often associated with current anti-diabetic treatment strategies, efforts to develop new pharmacological agents with better efficacy and fewer side effects are warranted^[3–6].

Recently, the role of the incretin hormones, glucagon-like peptide-1 (GLP-1), and their deficiency in patients with T2DM have been explored and targeted for new therapies with novel mechanisms of action. GLP-1 is released from L cells in the intestine after meal intake and plays a key role in the regulation of insulin secretion and glucose homeostasis. It has multiple metabolic effects that would be desirable attributes of an anti-diabetic agent^[7], such as glucose-dependent stimulation of insulin and suppression of glucagon release^[8], slowing of gastric emptying and appetite suppression^[9, 10], stimulation of non-insulin-mediated glucose uptake^[11], and suppression of endogenous glucose production independent of pancreatic hormones^[12]. Administration of GLP-1 or its mimic agents (eg, exendin-4 or liraglutide) is appealing due to their remarkable glucose-lowering efficacy and low frequency of hypoglycemia. The GLP-1-based therapeutics also appear to decrease beta-

* To whom correspondence should be addressed.

E-mail yleng@mail.shcnc.ac.cn

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cell apoptosis and increase beta-cell proliferation^[13, 14], which raises the theoretical possibility of slowing the progression of T2DM, a therapeutic strategy that goes beyond those offered by the traditional antidiabetic drugs^[3,4,6].

Native GLP-1 has a very short plasma half-life (approximately 2 min) because intact GLP-1 (GLP-1[7–36] amide) is rapidly degraded to an inactive form (GLP-1[9–36] amide) by DPP-4, which cleaves two residues from the NH₂-terminal end of the peptide^[15]. Moreover, due to their peptidic nature, GLP-1 and its analogs must be administered parenterally to exert their therapeutic actions. In contrast, small molecule inhibitors of DPP-4 were discovered to leverage the antidiabetic effects of endogenous GLP-1 and could be administered orally. Several orally available specific inhibitors of DPP-4 have been described and have been reported to improve glucose metabolism in various animal models of type 2 diabetes^[16–21] and more recently in diabetic patients^[22–24]. As one of earliest reported DPP-4 inhibitors, vildagliptin (formerly known as LAF237) was shown to be a selective and orally effective DPP-4 inhibitor, which was able to augment insulin release and reduce glucose excursions during an oral glucose tolerance test (OGTT) in Zucker fatty (*fa/fa*) rats and fat-fed normal rats after single and multiple oral administrations^[25, 26].

SHR117887 is a novel DPP-4 inhibitor discovered by Jiangsu Hansoh Pharmaceutical Co, Ltd (Jiangsu, China) for the treatment of type 2 diabetes. It is structurally different from other DPP-4 inhibitors that are currently available or in late-stage clinical development. As a competitive human DPP-4 inhibitor, SHR117887 showed high inhibitory potency against DPP-4 with an IC₅₀ of 17 nmol/L, and good selectivity against human DPP-8 or DPP-9 with an IC₅₀ of 4.51 μmol/L and 0.63 μmol/L, respectively (unpublished data supplied by Jiangsu Hansoh Pharmaceutical Co, Ltd). In the present study, we have characterized the acute *in vivo* effects of SHR117887 on blood glucose value, serum DPP-4 activity, insulin and active GLP-1 profiles after oral glucose loading in normal mice, diet-induced obese (DIO) rats and *ob/ob* mice. Moreover, the chronic administration of SHR117887 on metabolic control and pancreatic β-cell function in *ob/ob* mice was investigated and compared with LAF237, an approved anti-diabetic drug based on DPP-4 inhibition.

Materials and methods

Chemicals

SHR117887 (5-[2-(2-Cyano-4-fluoro-pyrrolidin-1-yl)-2-oxo-

ethylamino]-5-methyl-hexahydrocyclopenta[c]pyrrole-2-carboxylic acid dimethylamide *p*-toluenesulfonate, Figure 1) and vildagliptin (LAF237) were synthesized by Jiangsu Hansoh Pharmaceutical Co, Ltd (Jiangsu, China).

Animals

Male ICR mice and Wistar rats were purchased from the Shanghai SLAC Laboratory Animal Co Ltd (Shanghai, China). B6.V-*Lep^{ob}/Lep^{ob}* (*ob/ob*) mice and their lean littermates^{+/+} (from Jackson Laboratory, Bar Harbor, ME, USA) were bred at the Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences. The animals were maintained under a 12-h light-dark cycle with free access to water and food. Animal experiments were approved by the Animal Care and Use Committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Acute effect of SHR117887 on normal mice

To examine the acute effect of SHR117887 on blood glucose and serum DPP-4 activity after an oral glucose challenge, 0.1, 0.3, 1, or 3 mg/kg (0.186, 0.558, 1.86, and 5.58 μmol/kg) of SHR117887, 0.06, 0.19, 0.6, and 1.9 mg/kg of LAF237 (0.186, 0.558, 1.86, and 5.58 μmol/kg) or vehicle (distilled water) was orally administered to 5 h-fasted ICR mice (*n*=10 in each group) 60 min prior to the oral glucose load (2.5 g/kg). Blood samples were collected 60 min before the glucose load, and at 0, 15, 30, 60, 120, and 240 min after the glucose load in order to measure serum glucose and DPP-4 activity. To examine the acute effect of SHR117887 on the serum active GLP-1 level, blood samples were collected 15 min after the glucose load and placed in Eppendorf tubes containing the DPP-4 inhibitor valine pyrrolidide (Linco Research, DPP4-010) with a final concentration of 1% blood samples and 25 mg/mL EDTA to measure serum active GLP-1[7–36 amide] levels.

Acute effect of SHR117887 on diet-induced obesity (DIO) rats

Four-weeks old male Wistar rats were fed with a high-fat diet (D12492; Research Diets with 60% kcal% fat, New Brunswick, NJ, USA) for approximately 6 weeks and then divided into 7 groups based on serum glucose and body weight (*n*=10 in each group). The normal diet-fed Wistar rats were used as the lean control.

The rats were cannulated for blood sampling. An indwelling catheter was inserted under anesthesia in the right jugular vein, and externalized at the nape of the neck. Body weights were monitored, and studies were performed only after the rats regained their pre-surgery body weights. The oral glucose tolerance test (OGTT) was performed 3 d after surgery in overnight-fasted, awake, freely moving rats. SHR117887 (1, 3, and 10 mg/kg), LAF237 (0.6, 1.9, and 6.3 mg/kg) or vehicle (distilled water) was orally administered to rats 60 min prior to the oral glucose load (2.0 g/kg). Blood samples were collected from the jugular catheter 60 min before the glucose load, and 0, 5, 10, 15, 30, 60, and 120 min after the glucose load to measure serum DPP-4 activity, glucose and insulin levels. To analyze active GLP-1 levels, blood samples were collected

Figure 1. Chemical structure of SHR117887.

at 0, 5, 10, 15, 30, and 60 min after the glucose load and placed in Eppendorf tubes containing the DPP-4 inhibitor valine pyroglutamate (Linco Research, DPP4-010) with a final concentration of 1% blood samples and 25 mg/mL EDTA.

Acute effect of SHR117887 on type 2 diabetic *ob/ob* mice

Seven-week old *ob/ob* mice were divided into 7 groups ($n=10$ in each group) based on 6 h fasting blood glucose, serum insulin level, and body weight. Ten wild-type littermates were set up as the lean control group. SHR117887 (3, 10, and 30 mg/kg), LAF237 (1.9, 6.3, and 19 mg/kg) or vehicle (distilled water) was orally administered to 6 h-fasted *ob/ob* mice and lean mice 60 min prior to the oral glucose load (2.5 g/kg). Blood samples were collected 60 min before the glucose load and at 0, 15, 30, 60, and 120 min after the glucose load to measure serum glucose and insulin levels. Serum DPP-4 activity was measured at 2, 4, 8, and 12 h post-dose.

Chronic anti-diabetic effect of SHR117887 on type 2 diabetic *ob/ob* mice

Seven-week-old *ob/ob* mice were divided into 7 groups ($n=11$ in each group) based on non-fasting and fasting blood glucose, serum insulin levels and body weight. Wild-type littermates were used as the lean control. SHR117887 (3, 10, and 30 mg/kg), LAF237 (1.9, 6.3, and 19 mg/kg) or vehicle (distilled water) was orally administered twice daily (at 8:30 AM and 8:30 PM) for 33 d. Fasting blood glucose, body weight and food consumption values were determined at 4-d intervals. After 33 d of treatment, blood samples were collected after 6 h of fasting after the last dose for blood glucose and serum lipid level measurement. The pancreases of animals in the SHR117887 (30 mg/kg), LAF237 (19 mg/kg) and control groups were isolated and fixed for immunohistochemical analysis. To determine whether SHR117887 exhibited tachyphylaxis, the serum DPP-4 activity of another ten *ob/ob* mice treated with 30 mg/kg SHR117887 was measured at 2, 4, 8, and 12 h after the morning dose on d 1, d 12, d 24, and d 32.

Immunohistochemical analysis

The pancreatic samples were fixed in 10% buffered formalin for 1 d and subsequently embedded in paraffin. Paraffin sections (3 μ m) were cut, deparaffinized, rehydrated and placed in 3% hydrogen peroxide for 10 min at room temperature. The sections were then heated twice for 15 min at 90°C in a microwave, rinsed with Tris-buffered saline with Tween 80 (TBS-T) twice for 5 min, and then blocked with 5% normal goat serum (Beijing Dingguo Reagent Co, Ltd, Beijing, China) for 45 min. The sections were then incubated with the primary antibody, which was a ready-to-use guinea pig polyclonal anti-insulin antibody (Cat#: 58916, Abcam, Cambridge, UK) overnight at 4°C. The sections were washed with TBS-T and blocked with 10% normal goat serum for 30 min. Bound antibody was detected using a ready-to-use rabbit polyclonal to guinea pig IgG H&L (Cat#: 105460, Abcam, Cambridge, UK) for 30 min. The sections were rinsed with TBS-T and developed for 15 min using DAB (Beijing Dingguo Reagent Co, Ltd, Beijing, China).

Finally, the slides were washed with TBS-T, counterstained with hematoxylin, dehydrated and mounted.

Sample handling and analysis

Blood glucose was measured using a glucometer [ONE TOUCHTM BASICTM plus Glucose Monitor (Lifescan, Milpitas, CA, USA)]. Serum glucose was determined with a glucose oxidase method (Shanghai Shensuo Reagent Co, Ltd, Shanghai, China). The serum insulin level was measured with a 96-well ultra-sensitive mouse insulin ELISA kit (Cat#: 90080, Crystal Chem Inc, Downers Grove, IL). Serum free fatty acid (NEFA) level was measured by enzymatic methods with a test kit (Cat#: 994-75409, Wako Chemicals GmbH, Neuss, Germany). The serum total cholesterol (CHOL) level was measured with a test kit (Wenzhou Dongoujinma Reagent Co, Ltd, Wenzhou, China). Serum triglyceride (TG) was measured with a test kit (Shanghai Rongsheng Reagent Co, Ltd, Shanghai, China). Serum DPP-4 activity was measured using a fluorometric assay with the substrate Gly-Pro-AMC, which is cleaved by an enzyme to release a fluorescent label^[25]. Briefly, 5 μ L of serum sample was added to 96-well plates, followed by the addition of 35 μ L of 80 mmol/L $MgCl_2$ in an assay buffer (25 mmol/L HEPES, 140 mmol/L NaCl, 1% BSA, pH 7.8). After 5 min of pre-incubation at room temperature, the enzymatic reaction was started with the addition of 10 μ L of assay buffer containing 0.1 mmol/L substrate (Gly-Pro-AMC, Sigma). Liberation of AMC was monitored continuously at excitation 380 nm and emission 460 nm every 3 min for up to 18 min in a 96-well plate. Fluorometric catalysis rates were determined from the linear portion of the curve of the increase in fluorescence and were calculated as the slope of the regression line determined from the line. DPP-4 was expressed as the percentage of each animal's baseline level (the value obtained immediately before compound administration).

Statistical analysis

All data are expressed as the mean $\pm$ SEM. The statistical analysis between the two groups was performed using an unpaired Student's *t*-test. $P<0.05$ was considered to be statistically significant.

Results

Effect of acute administration of SHR117887 on serum DPP-4 activity, active GLP-1 and glucose levels in normal mice

SHR117887 dose-dependently inhibited serum DPP-4 activity (Figure 2A). Overall, 3.0 mg/kg (5.58 μ mol/kg) of SHR117887 achieved at least 70% DPP-4 inhibition throughout a 3 h period, which is similar to the inhibition achieved by the same molarity of LAF237. The ED_{50} value of SHR117887 for inhibition of serum DPP-4 activity was 0.69 mg/kg (1.28 μ mol/kg) at 1 h post-dose, while the ED_{50} value of LAF237 was 0.35 mg/kg (1.03 μ mol/kg). SHR117887 enhanced glucose-induced increases of active GLP-1 level, with the minimum effective dose of 0.3 mg/kg (Figure 2B). SHR117887 3 mg/kg raised the active GLP-1 level 2.7-fold compared with the vehicle control, and this effect was similar to that of 1.9 mg/kg LAF237

Figure 2. Effect of acute administration of SHR117887 on serum DPP-4 activity (A), active GLP-1 (B) and glucose levels (C, D) in normal mice. SHR117887, LAF237 or vehicle was orally administered to 5 h-fasted normal ICR mice 60 min prior to oral glucose load (2.5 g/kg). $n=10$. Data are mean $\pm$ SEM. ^b $P<0.05$, ^c $P<0.01$ vs control.

(2.8-fold *vs* the vehicle control). SHR117887 administration caused a significant decrease in glucose AUC_{0-120 min} by 36.6%, 44.5%, and 54.6% at the dose of 0.3, 1, and 3 mg/kg ($P<0.01$), respectively, while LAF237 showed a similar glucose-lowering effect with the reduction rate of 43.8%, 51.0% and 66.3% at the equimolar doses (Figures 2C and 2D), respectively. Therefore, a single oral dose of SHR117887 inhibited serum DPP-4 activity, increased serum active GLP-1 levels and improved glucose tolerance in normal mice after a glucose challenge.

Effect of acute administration of SHR117887 on serum DPP-4 activity, active GLP-1, glucose, and insulin levels in DIO rats

To generate a metabolic rodent model mimicking human type 2 diabetes with insulin resistance, Wistar rats were fed a high-fat diet. A single oral administration of SHR117887 inhibited the serum DPP-4 activities in a dose-dependent manner. Then, 10 mg/kg (18.6 μ mol/kg) of SHR117887 produced 88.3% inhibition on serum DPP-4 activity at 3 h post-dose, whereas 6.3 mg/kg (18.6 μ mol/kg) of LAF237 showed a similar effect (Figure 3A). The active GLP-1 level reached the peak value at 10 min after the oral glucose load in DIO rats, and 10 mg/kg SHR117887 raised the peak serum GLP-1 level by 4.9-fold and GLP-1 AUC_{0-60 min} by 5.6-fold compared with the vehicle control ($P<0.01$), while the same molarity of LAF237 raised the peak serum GLP-1 level and GLP-1 AUC_{0-60 min} by 4.9- and 5.8-fold ($P<0.01$), respectively (Figure 3B–3D). As shown in Figures 3E to 3G, DIO rats showed impaired glucose tolerance in response to an oral glucose challenge, while administration of

SHR117887 or LAF237 to DIO rats 1 h before the oral glucose load produced a significant decrease in glucose excursion. SHR117887 at the dose of 1, 3, and 10 mg/kg reduced glucose AUC_{0-120 min} values by 25.9%, 21.4%, and 42.8% ($P<0.01$, 0.05, 0.001), respectively, while LAF237 showed a comparable effect with decreases of 22.1%, 28.3%, and 44.2% ($P<0.05$, 0.01, 0.001), respectively. As shown in Figures 3H to 3J, administration of SHR117887 to DIO rats produced significant increases in insulin levels induced by the oral glucose challenge ($P<0.01$). At the dose of 10 mg/kg, SHR117887 treatment raised the insulin peak value by 2.0-fold compared with the vehicle control, and this effect was similar to that of 6.3 mg/kg of LAF237 (2.1-fold *vs* vehicle control). At the dose of 10 mg/kg, SHR117887 also raised the insulin AUC_{0-60 min} by 1.7-fold compared to the vehicle control ($P<0.05$), with a comparable effect of LAF237 at the dose of 6.3 mg/kg (2.0-fold *vs* the vehicle control, $P<0.01$).

Effect of acute administration of SHR117887 on serum glucose, insulin levels and DPP-4 activity in *ob/ob* mice

As shown in Figure 4, genetic type 2 diabetic *ob/ob* mice showed hyperglycemia, hyperinsulinemia and impaired glucose tolerance in response to oral glucose challenges. Single oral dose administration of SHR117887 reduced serum glucose levels and glucose AUC_{0-120 min} in a dose-dependent manner following an OGTT, and the efficacy was similar to that achieved by the same molarity of LAF237 (Figures 4A and 4B). At the oral dose of 3, 10, and 30 mg/kg (5.58, 18.6, and 55.8 μ mol/kg), SHR117887 reduced the glucose AUC_{0-120 min}

Figure 3. Effect of acute administration of SHR117887 on serum DPP-4 activity (A), active GLP-1 (B, C, D), glucose (E, F, G), and insulin levels (H, I, J) in DIO rats. SHR117887, LAF237 or vehicle was orally administered to overnight-fasted rats 60 min prior to oral glucose load (2 g/kg). $n=10$. Data are mean $\pm$ SEM. ^b $P<0.05$, ^c $P<0.01$ vs Control.

Figure 4. Effect of acute administration of SHR117887 on serum glucose (A, B), insulin levels (C, D) and DPP-4 activity (E) in *ob/ob* mice. SHR117887, LAF237 or vehicle was orally administered to 6 h-fasted *ob/ob* mice and their lean control mice 60 min prior to oral glucose load (2.5 g/kg). $n=10$. Data are mean $\pm$ SEM. ^b $P<0.05$, ^c $P<0.01$ vs Control.

by 30.2%, 35.0%, and 40.6%, respectively, while the reduction caused by the same molarity of LAF237 was 37.1%, 40.1%, and 48.4%, respectively (Figure 4B). SHR117887 also enhanced glucose-induced increases in serum insulin levels and insulin AUC_{0-60 min} in a dose-dependent manner (Figure 4C and 4D). At doses of 3, 10, and 30 mg/kg, SHR117887 increased insulin AUC_{0-60 min} by 2.6-, 3.0-, and 3.3-fold compared with the vehicle *ob/ob* control, while LAF237 was equally potent with increases

of 2.7-, 3.2-, and 3.4-fold, respectively (Figure 4D). As shown in Figure 4E, the serum DPP-4 activity was time- and dose-dependently reduced after SHR117887 or LAF237 administration, and the inhibitory effect on serum DPP-4 activity produced by 10 and 30 mg/kg SHR117887 at 8 h post-dose was 72.4% and 75.8%, respectively, which was similar to those of 6.3 and 19 mg/kg doses of LAF237 (73.6% and 83.5%, respectively).

Chronic anti-diabetic effect of SHR117887 in type 2 diabetic *ob/ob* mice

To evaluate the effects of chronic administration of SHR117887 on glucose metabolism and pancreatic β -cell function, 7-week old *ob/ob* mice were treated with SHR117887 (3, 10, and 30 mg/kg), LAF237 (1.9, 6.3, and 19 mg/kg) or vehicle alone twice daily for 33 d. As shown in Table 1, the fasting blood glucose values of *ob/ob* mice were significantly higher than those of lean mice during the entire treatment. Administration of SHR117887 with the dose of 10 and 30 mg/kg caused significant reductions in fasting blood glucose levels, and the average reduction during the entire treatment period was 35.3% and 31.8%, respectively, which was comparable to those of 6.3 and 19 mg/kg doses of LAF237 (31.5% and 35.6%, respectively). Chronic administration of SHR117887 also significantly improved the lipid homeostasis by reducing the serum triglyceride ($P<0.01$) and free fatty acid levels ($P<0.05$) in *ob/ob* mice, but it had no significant effect on total cholesterol levels (Table 2). SHR117887 caused a tendency of reduced food consumption, but had no effect on body weight (Table 2).

To determine whether SHR117887 exhibited tachyphylaxis, serum DPP-4 activity in ten *ob/ob* mice administered with 30 mg/kg SHR117887 was measured at 2, 4, 8, and 12 h after the morning dose on d 1, 12, 24, and 32. As shown in Figure 5, the profiles of serum DPP-4 activity on d 1, 12, 24, and 32 were

Figure 5. Serum DPP-4 activity profile in *ob/ob* mice treated with 30 mg/kg SHR117887 twice daily by oral administration for 32 days. $n=10$. Data are mean $\pm$ SEM.

virtually superimposable, suggesting that the chronic administration of SHR117887 exhibited no tachyphylaxis on the inhibition of DPP-4 activity.

At the end of study, the pancreases of the SHR117887 (30 mg/kg), LAF237 (19 mg/kg), vehicle control and the lean control mice were isolated and analyzed by immunohistochemistry using an anti-insulin antibody. As shown in Figure 6, weak insulin staining with irregular distribution in the β -cells

Table 1. Fasting blood glucose changes in *ob/ob* mice following chronic treatment with SHR117887 or LAF237 (Bid, po). Data are mean $\pm$ SEM. $n=11$. ^b $P<0.05$, ^c $P<0.01$ vs control.

Group	Dose (mg/kg)	Fast blood glucose (mmol/L)									Mean reduction rate of serum glucose (%)
		Pre-dose	d 4	d 8	d 12	d 16	d 20	d 24	d 28	d 33	
Lean mice	–	6.6 $\pm$ 0.2	6.0 $\pm$ 0.2	5.9 $\pm$ 0.3	6.0 $\pm$ 0.3	6.4 $\pm$ 0.3	5.7 $\pm$ 0.1	6.3 $\pm$ 0.2	6.4 $\pm$ 0.3	5.9 $\pm$ 0.2	–
Control	–	15.1 $\pm$ 1.1	20.1 $\pm$ 2.2	21.3 $\pm$ 1.8	20.5 $\pm$ 1.7	19.8 $\pm$ 2.3	21.6 $\pm$ 2.2	20.1 $\pm$ 2.0	20.7 $\pm$ 2.3	20.4 $\pm$ 1.7	–
SHR117887	3	15.2 $\pm$ 1.3	14.9 $\pm$ 2.4	15.6 $\pm$ 2.2	17.8 $\pm$ 2.6	15.6 $\pm$ 2.2	17.6 $\pm$ 2.8	15.2 $\pm$ 2.0	15.8 $\pm$ 2.3	14.6 $\pm$ 2.7	22.8 $\pm$ 1.8
	10	15.1 $\pm$ 0.9	13.1 $\pm$ 1.6 ^b	16.4 $\pm$ 1.3 ^b	14.1 $\pm$ 2.0 ^b	13.9 $\pm$ 2.0	13.9 $\pm$ 2.5 ^b	13.0 $\pm$ 2.1 ^b	11.6 $\pm$ 1.8 ^c	10.6 $\pm$ 1.5 ^c	35.3 $\pm$ 2.8
	30	15.1 $\pm$ 0.9	16.7 $\pm$ 1.5	14.8 $\pm$ 1.3 ^c	12.0 $\pm$ 1.4 ^c	14.1 $\pm$ 1.5 ^b	15.3 $\pm$ 1.3 ^b	12.7 $\pm$ 1.6 ^c	12.4 $\pm$ 1.8 ^b	14.0 $\pm$ 2.6 ^b	31.8 $\pm$ 2.7
LAF237	1.9	15.0 $\pm$ 1.4	15.7 $\pm$ 2.1	14.7 $\pm$ 1.7 ^b	13.3 $\pm$ 1.7 ^c	14.9 $\pm$ 1.6	14.6 $\pm$ 2.0 ^b	12.8 $\pm$ 1.8 ^b	14.9 $\pm$ 2.0	14.4 $\pm$ 2.6	29.7 $\pm$ 1.7
	6.3	15.1 $\pm$ 1.2	14.3 $\pm$ 1.6 ^b	14.0 $\pm$ 1.3 ^c	17.2 $\pm$ 2.1	17.3 $\pm$ 2.4	12.3 $\pm$ 1.5 ^c	12.4 $\pm$ 1.4 ^c	12.7 $\pm$ 1.6 ^b	12.2 $\pm$ 2.0 ^c	31.5 $\pm$ 4.0
	19	15.2 $\pm$ 1.1	14.8 $\pm$ 2.0	16.5 $\pm$ 2.3	15.3 $\pm$ 2.7	12.8 $\pm$ 2.2 ^b	12.6 $\pm$ 2.5 ^b	11.2 $\pm$ 1.9 ^c	11.1 $\pm$ 2.0 ^c	11.6 $\pm$ 2.3 ^c	35.6 $\pm$ 3.4

Table 2. Metabolic parameters in *ob/ob* mice following chronic treatment with SHR117887 or LAF237 for 33 days (Bid, po). $n=11$. Data are mean $\pm$ SEM. ^b $P<0.05$, ^c $P<0.01$ vs control.

Parameter (unit)	Control	<i>ob/ob</i> mice						Lean mice
		3 mg/kg SHR117887	10 mg/kg SHR117887	30 mg/kg SHR117887	1.9 mg/kg LAF237	6.3 mg/kg LAF237	19 mg/kg LAF237	
Serum NEFA (mmol/L)	1.4 $\pm$ 0.1	1.2 $\pm$ 0.1	1.1 $\pm$ 0.1 ^b	1.3 $\pm$ 0.1	1.1 $\pm$ 0.1 ^c	1.2 $\pm$ 0.1 ^b	1.1 $\pm$ 0.04 ^c	0.9 $\pm$ 0.1
Serum total cholesterol (mmol/L)	5.4 $\pm$ 0.3	5.2 $\pm$ 0.1	5.5 $\pm$ 0.3	5.6 $\pm$ 0.3	5.8 $\pm$ 0.3	4.8 $\pm$ 0.3	5.1 $\pm$ 0.4	1.9 $\pm$ 0.1
Serum triglyceride (mmol/L)	126.6 $\pm$ 5.8	97.3 $\pm$ 5.6 ^c	83.6 $\pm$ 3.9 ^c	100.6 $\pm$ 5.9 ^c	89.5 $\pm$ 5.5 ^c	101.4 $\pm$ 9.6 ^c	93.4 $\pm$ 4.9 ^c	82.4 $\pm$ 2.6
Food consumption (g/d)	7.5 $\pm$ 0.2	6.6 $\pm$ 0.2 ^c	7.0 $\pm$ 0.2	7.0 $\pm$ 0.3	6.8 $\pm$ 0.3	6.9 $\pm$ 0.3	6.7 $\pm$ 0.2 ^b	3.1 $\pm$ 0.0
Body weight (g)	56.2 $\pm$ 0.8	54.2 $\pm$ 0.9	55.6 $\pm$ 0.8	56.3 $\pm$ 1.2	55.4 $\pm$ 1.0	56.0 $\pm$ 1.0	55.5 $\pm$ 1.0	23.4 $\pm$ 1.1

Figure 6. Effects of chronic SHR117887 treatment on pancreatic insulin staining in *ob/ob* mice. Mice were treated with SHR117887 (30 mg/kg), LAF237 (19 mg/kg) or vehicle twice daily by oral administration for 33 days. Pancreata were isolated and pancreatic sections were stained with anti-insulin antibodies after 6 h fasting. (A) Vehicle-treated lean mice. (B) Vehicle-treated *ob/ob* mice. (C) 30 mg/kg SHR117887-treated *ob/ob* mice. (D) 19 mg/kg LAF237-treated *ob/ob* mice. $\times 100$.

was observed in the vehicle-treated *ob/ob* mice compared with the vehicle-treated lean mice, which is indicative of impaired β -cell function in the *ob/ob* mice. The 33-d treatment with SHR117887 significantly increased insulin staining and enhanced insulin antigen positivity with regular distribution in β -cells, indicating the improved β -cell function. Chronic treatment with the dose of LAF237 with similar plasma exposure showed a similar effect.

Discussion

Inhibition of DPP-4 augments the level of active GLP-1 by inhibiting the degradation, and it returns glucose homeostasis toward physiological control levels^[27]. Therefore, DPP-4 inhibitors are expected to become a new class of anti-hyperglycemic drugs. SHR117887 is a novel potent DPP-4 inhibitor, which is currently in a phase I clinical study. In the present research, we report the acute and chronic pharmacological effects of SHR117887 on DPP-4 inhibition and metabolic control in normal or diabetic rodent models. Moreover, the efficacy of SHR117887 was compared with LAF237 at the same molar dose in each experiment.

In normal mice, acute administration of SHR117887 exhibited good oral bioavailability and caused dose-dependent inhibition of serum DPP-4 activity, accompanied with enhanced active GLP-1 levels and decreased serum glucose values. The minimum effective dose of SHR117887 to augment active GLP-1 and reduce glucose excursions was 0.3 mg/kg, which caused a 27.8% increase of active GLP-1 level and a 36.6% reduction of glucose AUC_{0–120 min}. The DPP-4 activity was inhibited by 30% to 40% in the 2 h after a single oral dose of 0.3 mg/kg (0.558 μ mol/kg) of SHR117887, suggesting that

approximately 40% inhibition of serum DPP-4 activity is sufficient for effectiveness in oral glucose challenge in normal mice, which might be contributed by the normal whole body insulin sensitivity in ICR mice. LAF237 showed comparable efficacy at the same equimolar doses, and the minimum effective dose of LAF237 to augment active GLP-1 and reduce glucose excursions was 0.19 mg/kg (0.558 μ mol/kg).

T2DM is often characterized by insulin resistance and is associated with diet-induced obesity and impaired glucose tolerance. We therefore further investigated the acute pharmacological effect of SHR117887 in the DIO rat, a model with modest insulin resistance and glucose intolerance^[28]. The minimum effective dose of SHR117887 to augment the peak value of active GLP-1 and GLP-1 AUC_{0–60 min} was 3 mg/kg, which is also the minimum effective dose to increase the insulin peak value in 15 min after glucose loading. The acute administration of 3 mg/kg of SHR117887 could inhibit serum DPP-4 activity by 70% in the 2 h after dosing, but caused only a mild reduction of blood glucose AUC_{0–120 min} by 21.4%, which suggested that more powerful inhibition of DPP-4 activity was necessary to achieve effective glucose excursions reduction in insulin resistant animal models.

The acute glucose-lowering effect of SHR117887 was further evaluated in *ob/ob* mice, a genetic type 2 diabetic rodent model that exhibits hyperglycemia, hyperinsulinemia, hyperglucagonemia, and severe whole body insulin resistance^[29]. A single oral dose of 3, 10, and 30 mg/kg SHR117887 inhibited serum DPP-4 activity by 84%, 91%, and 93%, respectively, at 2 h post-dose, reducing serum glucose AUC_{0–120 min} by 30.2%, 35.0%, and 40.6%, respectively. LAF237 showed a similar pharmacological effect to SHR117887, and these findings are consistent with the previous reports using other DPP-4 inhibitors in glucose-intolerant rodents, including high-fat-fed rats^[26, 28], Zucker fatty rats^[30, 31] and mice^[32]. These results suggest that SHR117887 improves glucose tolerance through the elevation of serum insulin and active GLP-1 levels via the inhibition of DPP-4 activity in normal and diabetic animal models, but the achievable glucose lowering effect seems to be correlated with the degree of insulin resistance in different animal models.

Several studies have demonstrated the effectiveness of long-term DPP-4 inhibition on amelioration of metabolic disorder in diabetic animal models^[20, 33]. In the present study, the anti-diabetic effects of chronic DPP-4 inhibition by SHR117887 were investigated in *ob/ob* mice. Because the acute study showed that a single oral dose of 3, 10, and 30 mg/kg of SHR117887 in *ob/ob* mice can only inhibit serum DPP-4 activity by 5.1%, 23.9%, and 52.8%, respectively, at 12 h post-dose, a twice-daily administration was chosen for the chronic study to maintain adequate inhibition of DPP-4 activity throughout the experiment. The dose of 30 mg/kg SHR117887 caused 88.9% and 46.6% inhibition of serum DPP-4 activity at 2 h and 12 h post dose, whereas administration of the same dose of SHR117887 on day 12, 24, and 32 of the chronic treatment exerted almost equivalent DPP-4 inhibition, which indicated that SHR117887 does not exhibit tachyphylaxis when given orally twice daily for 32 d. In agreement with previous stud-

ies of the DPP-4 inhibitor^[16, 20], SHR117887, significantly decreased fasting blood glucose values and the results were also observed at a comparable level with the same molar dose of LAF237. Moreover, lipid homeostasis was also improved by chronic SHR117887 treatment in *ob/ob* mice by reducing the serum triglyceride and NEFA levels, which is consistent with the results of previous studies conducted with alogliptin on *ob/ob* and *db/db* mice or with a sitagliptin analog (des-fluoro-sitagliptin) in high-fat diet streptozotocin-induced diabetic mice^[34-36], suggesting possible beneficial effects of SHR117887 in type 2 diabetic patients associated with lipid dysregulation.

GLP-1 and its analogs have been demonstrated to reduce food intake and decrease the body weight in diabetic animal models and clinical studies^[37-39]. Inhibition of DPP-4 activity prevented degradation and enhanced the biological activity of active GLP-1^[15]. However, chronic administration of SHR117887 showed only a minor tendency in the reduction of food consumption and had no effect on body weight. These results are similar to the reports of several other studies of DPP-4 inhibitors^[34, 35], and the possible reasons might be due to the less effect of the enhanced endogenous active GLP-1 caused by DPP-4 inhibition rather than that of the injection of exogenous GLP-1 or its analogs.

A great challenge in the therapy of type 2 diabetes patients is the progressive loss of β -cell mass and deterioration of β -cell function^[2, 3]. In the present study, chronic treatment with SHR117887 increased the insulin staining in islet cells, suggesting improved β -cell function. Because GLP-1 has been reported to decrease β -cell apoptosis and increase β -cell proliferation^[13, 14], the improved β -cell function caused by SHR117887 might be due to the enhanced and prolonged endogenous GLP-1 action after long-term inhibition of DPP-4 activity. Moreover, the improvement of glucose control and decreased triglyceride and NEFA levels probably also contributed to the beneficial effects of the chronic administration of SHR117887 on β -cell function in *ob/ob* mice.

In conclusion, this study has demonstrated that SHR117887 is a potent DPP-4 inhibitor, which increases active GLP-1 and insulin levels and improves glucose homeostasis after acute dosing in normal, non-genetic and genetic-type 2 diabetes animal models. Chronic treatment with SHR117887 improves glycemic control, decreases serum triglyceride and NEFA levels, and improves β -cell function in type 2 diabetes *ob/ob* mice. These findings suggest the usefulness of SHR117887 for further development as a new therapeutic agent for impaired glucose tolerance and type 2 diabetes. SHR117887 is currently in phase I clinical studies.

Author contribution

Ying LENG designed the research; Xiao LIU, Li-na ZHANG, Ying FENG, Lei ZHANG, and Hui QU performed the research; Xiao LIU, Ying FENG, and Guo-qing CAO analyzed the data; Xiao LIU, Guo-qing CAO, and Ying LENG wrote the paper.

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