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DBPR108, A Dipeptidyl Peptidase-4 Inhibitor for Treatment of Type 2 Diabetes Mellitus: A Phase I, Randomized, Double-Blind, Placebo-Controlled Trial of Single and Multiple Doses in Healthy Volunteers

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1. Abstract

1.1. Aims

DBPR108 is an orally active dipeptidyl peptidase 4 (DPP-4) inhibitor in animals. The present first-in-human randomized, double-blind, and placebo-controlled study evaluated DBPR108 for safety and tolerability in healthy adult Asian male volunteers.

1.2. Main Methods

DBPR108 were orally given at 25-600 mg as single and multiple once-daily doses for 8 consecutive days. Hospital stays and visits were scheduled for oral ingestion of DBPR108 and physical examinations including vital signs, clinical laboratory tests, and electrocardiograms throughout the study. Blood and urine samples were collected for pharmacokinetic and pharmacodynamic measurements, including DPP-4 activities, and active glucagon-like peptide-1 (GLP-1) levels.

1.3. Significance

DBPR108 is readily oral absorbable and inhibits the circulating DPP-4 activity in healthy volunteers. DBPR108 is well-tolerated without safety concerns of hypoglycemia or acute pancreatitis. The Phase 1 study results warrant further investigations of DBPR108 for treating T2DM patients.

1.4. Clinical Trial Identifiers: NCT01650324 and NCT02163278

2. Introduction

Diabetic mellitus has been a major disease of global prevalence for more than three decades. Type 2 diabetic mellitus (T2DM) is a chronic metabolic disorder diagnosed with insulin resistance, reduced glucose tolerability and chronic fasting hyperglycaemia, leading to serious systemic damages to the blood vessels, nerves, eyes, heart and kidneys. A global prevalence of 570.9 million diabetes patients and

1.59 million deaths were estimated in 2025 [1] and an increased global prevalence to 700 million patients by 2045 [2]. Similarly, the diabetes prevalence in the United States has also been increasing since 1999 till recent statistics of more than 34 million diabetes Americans, accounting for 10.5% of the population in which approximately 90-95% are T2DM patients [3]. Glucagon-like peptide-1 (GLP-1) is an incretin released, upon food ingestion, from intestinal endocrine L-cells of the lower intestine and colon [4], and increased to an approximate 2-5 fold in circulation [5]. GLP-1 is essential for maintaining the glucose homeostasis. However, the circulation half-life of GLP-1 is short within minutes [6] due to its renal excretion and enzymatic degradation/inactivation by the circulating serine protease, dipeptidyl peptidase 4 (DPP-4; CD26) [7]. With the short half-life, GLP-1 itself is not feasible as therapeutics [8], but promising as a target for discovery of diabetes-curing drugs. Discovery strategies of diabetes-curing drugs as to maintain or increase the pharmacological activities and circulating levels of active GLP-1 have been explored, such as GLP-1-mimetics, GLP-1 receptor agonists [9] and DPP-4 inhibitors [10,11]. Concerns pertaining to GLP-1-mimetics, liraglutide and exenatide, are that they must be given in injectable form [12] and adverse effects (pancreatitis and others) have been reported [9, 13-15]. An orally absorbable GLP-1 receptor agonist is in clinical development from which clinical benefits shall be followed [16]. DPP-4 is widely expressed throughout the body [17] in membrane-bound and soluble form in the circulation with GLP-1-degrading enzyme activity [18]. To inhibit the GLP-1-degrading activity of DPP-4 and thus to prolonging the circulating half-life of active GLP-1 has been a valid therapeutic strategy for treating T2DM [11]. Several DPP-4 inhibitors are available in the United States, European Union, Japan and South Korea [10, 19, 20]. In general, these DPP-4 inhibitors showed antidiabetic efficacies and good tolerability. However, clinical adverse events were noted as increased risks of prostate cancer, bile duct stone, acute pancreatitis, infections, inflammatory bowel disease, anaphylactoid reaction, angioedema, exfoliate dermatitis and severe joint pain [21-25]. DBPR108 is a synthetic small molecule with potent (IC_{50}=5-15 nM) activity inhibiting DPP-4 enzyme with excellent selectivity over the other prolyl-cleaving proteases [26]. DBPR108 is orally absorbable in mice, rats, and dogs and showed significant activities dose-dependently inhibiting the plasma DPP-4 in these animals [27]. Furthermore, DBPR108 increased the circulating active GLP-1 levels, prolonged its systemic exposure time, and thus improved oral glucose tolerability in animals [27]. DBPR108 is a drug candidate currently being developed in clinical studies for treating T2DM. We reported here the pharmacokinetics, pharmacodynamics, safety and tolerability of DBPR108 orally given at single and repeated doses in healthy male Asian volunteers from a Phase 1 clinical study

3. Study Design and Methodologies

3.1. Study Design

The double-blinded, randomized, placebo-controlled Phase 1 study was conducted in two, single-dose and multiple-dose, phase studies (**Clinical trial identifiers:** NCT01650324 and NCT02163278) sequentially at the WanFang Hospital, Taipei and the Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, respectively. For the two-phase studies, 4 dose cohorts per phase were randomly assigned with the adult healthy Asian male volunteers recruited at 8 (6 DBPR108-treated and 2 placebo-treated) subjects for each dose cohort. The dose levels of the 4 DBPR108-treated cohorts are 25, 100, 300 and 600 mg in both single- and multiple-dose phase studies. In the single-dose study phase, there were 3 hospital visits and 1 hospital staying period, i.e., a screening visit, a hospital residential period (Day -1 to 3), a follow-up visit on Day 5, and an end-of-study visit on Day 7 as shown in Figure 1A. The lowest dose of DBPR108 was firstly given to subjects in Cohort 1 and the safety and tolerability of the lower doses were evaluated and determined to be acceptable before proceeding to the next higher dose level. Between each dose level escalation to a different cohort, there was a safety evaluation period of at least 1 week. Within a given cohort, each subject received a single oral dose of DBPR108 or placebo double-blinded according to their assigned randomization under fasting condition on Day 1 followed by collections of blood and urine for pharmacokinetic measurements and blood sampling up to 48 h after the oral dose given for pharmacodynamic evaluations. Safety was assessed up to 6 days after dosing. In the multiple-dose study phase, there were 7 hospital visits and 2 hospital staying periods, i.e., a screening period (Days -21 to Day -4, Visit 1), the first hospital residential period (Day -1 to Day 3, Stay 1), 4 treatment visits on the mornings of Day 4 to Day 7 (Visit 2 to Visit 5), the second residential period (Day 7 to Day 10, Stay 2), a follow-up visit (Day 11, Visit 6), and an end-of-study visit on Day 12 (Visit 7) as shown in Figure 1B. The lowest dose of DBPR108 was given to subjects in Cohort 1. Escalation to the next dose level was based on interim safety and tolerability data of the previous dose level. Within a given cohort, each subject received once daily oral doses of DBPR108 or placebo double-blinded in the morning of Days 1 to 8, respectively, according to their assigned randomization. For each subject, blood samples were collected from Days 1 to 12; a total of 6 pharmacokinetic urine samples were collected up to 24 h after the first dose (Day 1) and up to 48 h after the last dose (Day 8); and pharmacodynamic blood samples were collected up to 48 h after the first and last doses on Days 1 and 8. During the hospital staying periods, 4 standardized test meals (breakfast and dinner on Days 1 and 8) were served to evaluate the food effect on the pharmacokinetics and pharmacodynamics of DBPR108.

3.2. Inclusion Criteria

Eligible volunteer subjects who provided a written informed consent were nonsmoking male, aged between 20 and 45 years, body weight >50 Kg and/or BMI ≧18.5 and <24 Kg/m^2, with normoglycemic (fasting glucose at ≧70 and <100 mg/dL). Subjects were included in the study in good general health based on routine medical history, vital signs, physical examination, electrocardiography (ECG) and laboratory tests including hematology, biochemistry, urinalysis, serology screen, creatinine clearance, finger stick blood glucose test, habits of nicotine and alcohol use (serum alcohol screen), and urine drugs of abuse test. Those who used any antihyperglycemic agents at screening, had donated blood or participated in a clinical study within 8 weeks, excessive intake of caffeine-containing drinks or food, or used any prescription or nonprescription medication within 2 weeks or 5 half-lives, were excluded from the study.

3.3. Investigational Product DBPR108 and Medications

The investigational product DBPR108 formulated in capsules was prepared by a GMP/cPICS-certified company Sinphar Pharmaceutical Co., Ltd (Ilan, Taiwan). DBPR108 powders were accurately weighed into capsules at dosages of 25 and 100 mg per capsule. All investigational products of DBPR108 were swallowed in capsule(s) with 240 mL water without chewed or crushed at the study site under food and fluid restrictions as supervised by the study site staff. In each of the two study phases, Cohorts 1 to 4 are subjects administered with 25, 100, 300, and 600 mg of DBPR108, respectively. In single-dose phase study, each subject received a single oral dose of DBPR108 or placebo by randomly assigned on Day 1 after an overnight fasting of food and water for at least 10 h. After the DBPR108 ingestion, all subjects remained fasting for another 4 h without water intake. Being further explored, the food effect on the pharmacokinetic and pharmacodynamic profiles of DBPR108 was evaluated in the multiple-dose phase study, in which each subject was blinded to receive a total of 8 once daily oral doses of DBPR108 or placebo in the mornings of Days 1 to 8 after an overnight fast of at least 10 h. All subjects were served with 4 standardized test meals identical for all of the enrolled subjects as breakfast and dinner on Day 1 and Day 8. The breakfast test meals were given at approximately 5 min after the DBPR108 dosing in the mornings and the dinner test meals were given at approximately 10 h after the DBPR108 dosing on both Day 1 and Day 8. All test meals were consumed in full within 15 min. The standardized test meal was consisted of a nutrition breakdown of approximately 55% carbohydrate, 30% fat and 15% protein with an approximately 600±50 Kcal in each serving.

3.4. Pharmacokinetic Study

In single-dose phase study, 4 mL of blood samples were drawn on the first day of study (pre-dose, as 0-h) and at 0.5, 1, 1.5, 2, 4 (before lunch), 6, 8, 12, 16, 24, 48, 72, and 96 h after dosing. In multiple-dose phase study, 2 mL of blood samples were drawn at before dosing 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4 (before lunch), 6, 8, 12, and 16 h post the first dose on Day 1; before each dosing on Days 2 to 7; before dosing 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4 (before lunch), 6, 8, 12, 16 on Day 8 and extended to Day 12 for blood sample collections at 24, 36, 48, 72 and 96 h post the last dose. Urine samples of 3 mL were collected at 0 (pre-dose), 0 to 12 and 12 to 24 h post the first dose on Day 1; 0 to 12, 12 to 24, and 24 to 48 h post the last dose on Day 8. After centrifugation at 2000-4000×g for 10 min at 4°C. 0.5-1 mL of plasma were transferred into two tubes, separately. 0.5-1 mL of 20% trichloroacetic acid solution was added into the tubes for mixing. 5 mL of urine samples were collected after administration during intervals of 0 to 12, 12 to 24, and 24 to 48 h post-dose and 10 mL urine was transferred into tubes. The plasma and urine samples kept in tubes were stored at ≦-70°C until analyzed for DBPR108 concentrations using LC-MS/MS bioanalytical methods [27] validated and performed at the National Veterinary Institute, Department of Chemistry, Environment, and Feed Hygiene (Uppsala, Sweden) and QPS (Taipei, Taiwan) with an assay range of 2 to 1000 ng/mL for plasma and of 1 to 1000 ng/mL for urine. Pharmacokinetic parameters were determined by using noncompartmental methods with WinNonlin® Professional Version 5.2/6.3 from Pharsight Corp. (Mountain View, CA, USA) or SAS® version 9.2 from SAS Institute (Cary, NC, USA). Pharmacokinetic parameters estimated were area under the plasma concentration-time curve from time zero to infinity, $AUC_{0\text{-inf}}$; area under the plasma concentration-curve from time zero to the time of last quantifiable plasma concentration, $AUC_{0\text{-last}}$; observed maximum plasma concentration, C_{max}; time of maximum plasma concentration, T_{max}; apparent terminal plasma half-life, $T_{1/2}$; apparent plasma clearance following extravascular dosing, CL/F; apparent volume of distribution following extravascular dosing, V_z/F; cumulative amount of unchanged drug excreted in urine from time zero to time t; $Ae_{0\text{-t}}$; fraction of dose excreted into the urine from time zero to time t, $fe_{0\text{-t}}$; renal clearance, CL_R; and accumulation ratio, AR.

3.5. Measurement of Pharmacodynamic Profiles

In single-dose phase study, pharmacodynamic venous blood samples of 8 mL were drawn at pre-dose (as 0-h) and at 1, 2, 4 (before lunch), 8, and 16 h post-dose on Day 1; at 24 h post-dose on Day 2; and at 48 h post-dose on Day 3. In multiple-dose phase study, blood samples of 7 mL were drawn on Days 1 to 3 at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4 (before lunch), 6, 8, 10 (before dinner), 10.5, 11, 11.5, 12, 16, 24 and 48 h post the first dose; on Days 8 to 10 at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4 (before lunch), 6, 8, 10 (before dinner), 10.5, 11, 11.5, 12, 16, 24 and 48 h post the last dose for pharmacodynamic assays. Methods for measuring the pharmacodynamic parameters of plasma or serum DPP-4 activities, active GLP-1 levels, and all the others included were validated and measurements performed by Quintiles Laboratories, (Marietta, GA, United States), QPS (New Taipei City, Taiwan), or the 2 study hospitals. Assay kits such as a fluorometric DPP-4/CD26 assay kits from Enzo Life Sciences (Plymouth Meeting, PA, USA) for DPP-4 activity and a electrochemical chemiluminescence multi-array MSD® metabolic assay from Meso Scale Discovery (Rockville, MD,

USA) for active GLP-1 quantification were used.

3.6. Safety and Tolerability Assessments

Safety and tolerability assessment tests include clinical laboratory tests (hematology, blood chemistry, urinalysis, serology screen, creatinine clearance, finger stick blood glucose test, 12-lead ECGs, adverse events (including intolerable adverse events, serious adverse events, and adverse events of special interest such as hypoglycemia and pancreatitis), vital signs, and physical examinations. conducted in accordance with the study schedule. For vital signs examination, blood pressure, pulse, and body temperature were measured as scheduled after the subjects have rested in a supine position for 10 min. The normal ranges for systolic/diastolic blood pressure were from 90/60 to 140/90 mmHg, for pulse was from 45-90 beats/min, and for body temperature was from 35-37.5°C. Physical examinations included an examination of the following: general appearance, eyes, ears, nose, throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological status. All data pertaining to the date, time, and measurement values were monitored and entered into the case report form properly following the good clinical practice (GCP) compliance.

3.7. Statistical Analysis

The pharmacokinetic and pharmacodynamic data were summarized in study cohorts and statistically significant differences among study cohorts were analyzed. All subjects receiving placebo in separate cohorts were pooled into one single group for data comparisons. A p value of less than 0.05 was considered statistically different between groups after *ANOVA* analysis followed by the *Student-Newman-Keuls* multiple comparisons.

4. Results

4.1. Subject Screening, Demographics and Disposition

A total of 143 Asian male volunteers were screened for the two, single-dose and multiple-dose, study phases. Seventy-one volunteers were screened for the single-dose phase study and 37 subjects met the eligibility criteria. Among them, 4 subjects were excused from the study because continuing eligibility requirements failed and 1 subject was withdrawn from the study. The remaining 32 subjects were included and randomly assigned to a single dose treatment with DBPR108 or placebo. The 32 healthy male subjects included in the single-dose study had a mean age of 27 (ranged from 20 to 42) years, a mean height of 173.5 (ranged from 163.8 to 186.5) cm, a mean weight of 72.7 (ranged from 54.1 to 102.9) Kg, and a mean body mass index (BMI) of 24.0 (ranged from 18.1 to 30.0) Kg/m^2 (Table 1). In multiple-dose phase study, 72 healthy Asian male volunteers were screened and 32 subjects met the eligibility criteria; 40 subjects failed to participate due to consent withdrawal, time commitment and ineligibility. The 32 subjects were randomly assigned to treatments and completed the study. They had a mean age of 30.4 (ranged from 21.0 to 41.6) years, a mean height of 173.1 (ranged from 162.6 to 182.2) cm, a mean weight of 66.9 (ranged from 53.1 to 78.5) Kg, and a mean BMI of 22.3 (ranged from 19.6 to 23.9) Kg/m^2 (Table 1). All 64 recruited eligible and healthy male volunteers were enrolled randomly in the 4 cohorts, respectively, in the 2 study phases implemented with the study protocols accordingly as the study scheme indicated in Figure 1.

4.2. Pharmacokinetic Parameters Determined

After orally ingested, DBPR108 was rapidly absorbed in the healthy male volunteers and plasma DBPR108 concentrations increased dose dependently as shown in Figure 2A. Plasma and urinary DBPR108 pharmacokinetic parameters after single dose were summarized in Table 2. As noted in Table 2, both plasma C_{max} of DBPR108 and drug exposure in AUC(0-inf) increased in a significant dose dependent manner (Figure 2) with a median T_{max} reached at 1.9 to 4 h across the 4 dose levels. The plasma DBPR108 concentrations declined with a mean $T_{1/2}$ ranged from 3.1 to 18.6 h, CL/F from 59.6 to 113 L/h, and Vz/F from 499 to 2230 L; none of these 3 parameters showing a dose dependency. The mean renal clearance, CLR was 9.4 to 15.2 L/h across doses as shown in Table 2. In the regimen of multiple doses, DBPR108 was absorbed rapidly, similar to that observed in the single dose regimen, following the first oral doses of 25 to 600 mg on Day 1 in the healthy male subjects (Figure 2B). The plasma DBPR108 concentrations were able to be maintained in a dose dependent manner during the period of daily visits in which the once daily oral doses were administered. In general, a pharmacokinetic profile similar to that of the single-dose study (Figure 2A) was observed on Day 8 in the multiple-dose study (Figure 2B). Pharmacokinetic parameters of DBPR108 orally ingested at once daily multiple doses were summarized in Table 3. On Day 1, the median T_{max} ranged from 2.92 to 4.33 h after dosing and apparent terminal $T_{1/2}$ of the plasma DBPR108 concentration was similar over the dose range of 100 to 600 mg at 5.06 to 6.60 h, but relatively short for 25 mg at 2.28 h. Mean CL/F ranged from 68 to 142 L/h, and mean V_z/F ranged from 469 to 895 L. On Day 8, DBPR108 was still rapidly absorbed following multiple dosing with an observed median Tmax ranged from 1.92 to 3.17 h, which were not significantly different among the cohorts after having received multiple doses of 25 to 600 mg. The mean apparent terminal T1/2 of DBPR108 was similar over the dose range of 100 to 600 mg at 10.3 to 13.5 h, but relatively short for 25 mg at 4.61 h. The mean CL/F ranged from 62 to 139 L/h and mean V_z/F ranged from 863 to 2120 L were observed. It appeared that the plasma Cmax and AUC0-24/0-last of DBPR108 concentrations were increased in a dose proportional manner after the DBPR108 administrations followed by the measurements for plasma pharmacokinetic profiling (Figure 2) and showed a good linear relationship between them with an R value of 0.9655, 0.9458, and 0.9262 for Day 1 of single-dose, Day 1, and Day 8 of multiple-dose phase, respectively. The slopes (Day 1 of single-dose: 2.30; Day 1: 2.05 and Day 8: 1.85 of multiple-dose) of the 3 lines of linear regression were not different to each other. Assessment of AUC0-24h

accumulation ratios revealed a minimal accumulation over the 8-day study period. Mean accumulation ratios (AR), calculated by dividing the exposure AUC0-24h from Day 8 with the exposure from Day 1, ranged from 1.07 to 1.20 (Table 3). In relation to the oral dose ingested, the fraction of intact parental DBPR108 excreted in the urine unchanged over 24 h (fe0-24h) appeared to increase with dose after single and multiple doses of DBPR108 (Tables 2 and 3). The accumulated amount of the ingested DBPR108 dose excreted unchanged in the urine, $Ae_{0\text{-}24h}$ or $Ae_{0\text{-}48h}$ was estimated at less than 20% in both the single-dose and multiple-dose studies. The fe0-24h ranged from 8.9 to 19.2% in the single-dose study; from 5.7 to 16.0% on Day 1 and 5.6 to 18.9% on Day 8 in the multiple-dose study. Over the dose range of 25 to 600 mg DBPR108, mean CLR ranged from 9.4 to 15.2 L/h in the single-dose study; from 8.0 to 13.7 L/h and 7.4 to 15.0 L/h in on Day 8 in the multiple-dose study, respectively. There was a trend of dose related increment in CL_R from dose cohort of 25 mg up to 300 mg, with an observation of a lower CL_R in the 600 mg cohort than that in 300 mg cohort.

Table 1: Demographics and baseline characteristics of the Asian healthy male volunteers

Characteristics	DBPR108				
	Placebo (N=8)	**25 mg (N=6)**	**100 mg (N=6)**	**300 mg (N=6)**	**600 mg (N=6)**
Single Dose Study					
Age, years	31 ± 8 (22, 42)	26 ± 2 (22, 29)	24 ± 2 (22, 28)	27 ± 5 (23, 37)	26 ± 6 (20, 36)
Height, cm	170.5 ± 3.9 (165.0, 176.5)	175.0 ± 5.4 (169.4, 182.3)	171.3 ± 7.7 (163.8, 185.1)	177.0 ± 5.4 (170.6, 186.5)	174.7 ± 4.5 (168.2, 178.3)
Weight, Kg	71.4 ± 7.3 (62.2, 86.3)	69.5 ± 11.3 (54.1, 86.8)	71.9 ± 16.3 (54.9, 95.9)	76.2 ± 15.6 (54.9, 102.9)	74.9 ± 9.3 (65.2, 87.3)
BMI, Kg/m^2	24.5 ± 2.6 (21.2, 28.4)	22.6 ± 2.5 (18.6, 26.1)	24.3 ± 4.0 (20.4, 30.0)	24.1 ± 4.0 (18.1, 29.5)	24.5 ± 2.5 (21.5, 27.4)
Multiple Dose Study					
Age, years	32 ± 2 (30, 35)	29 ± 5 (21, 35)	30 ± 7 (22, 37)	32 ± 5 (26, 42)	29 ± 4 (22, 32)
Height, cm	172.1 ± 3.4 (167.8, 176.9)	172.2 ± 3.6 (167.3, 175.3)	172.1 ± 5.9 (162.6, 178.6)	174.8 ± 6.7 (165.2, 182.1)	174.6 ± 4.9 (169.5, 182.2)
Weight, Kg	68.4 ± 3.5 (64.0, 72.7)	64.3 ± 6.5 (55.3, 73.5)	64.6 ± 8.0 (53.1, 74.0)	67.8 ± 7.6 (59.5, 78.5)	68.7 ± 6.1 (58.9, 76.3)
BMI, Kg/m^2	23.1 ± 0.4 (22.6, 23.9)	21.6 ± 1.7 (19.6, 23.9)	21.7 ± 1.4 (20.1, 23.2)	22.1 ± 1.4 (20.5, 23.7)	22.5 ± 1.4 (19.7, 23.9)

Data are represented as mean ± S.D.; Minimum and maximum values are shown in the parenthesis.

Table 2: Pharmacokinetic parameters of DBPR108 after single oral administration in healthy human male subjects.

Parameter	Dose Cohort			
	25 mg	**100 mg**	**300 mg**	**600 mg**
$AUC_{0\text{-last}}$ (ng−h/mL)	225 ± 66	1,240 ± 302[a]	3,840 ± 1,220[ab]	10,400 ± 2,500[abc]
$AUC_{0\text{-inf}}$ (ng-h/mL)	236 ± 67	1,310 ± 290[a]	3,910 ± 1,200[ab]	10,500 ± 2,400[abc]
C_{max} (ng/mL)	47 ± 24	187 ± 33[a]	571 ± 158[ab]	1,370 ± 235[abc]
T_{max} (h)	1.9 ± 1.0	3.6 ± 1.0[a]	4.0 ± 2.0[a]	4.0 ± 1.8[a]
$T_{1/2}$ (h)	3.1 ± 0.4	18.6 ± 13.3[a]	7.7 ± 3.8[a]	12.6 ± 14.1
CL/F (L/h)	113.0 ± 29.4	79.7 ± 19.1[a]	88.1 ± 45.3	59.6 ± 14.3[a]
VZ/F (L)	499 ± 124	2,230 ± 2,100	1,050 ± 984	1,210 ± 1,520
$Ae_{0\text{-}24h}$ (mg)	2.2 ± 1.0	13.1 ± 6.5[a]	55.9 ± 26.3[ab]	115.0 ± 62.1[abc]
$fe_{0\text{-}24h}$ (%)	8.9 ± 3.9	13.1 ± 6.5	18.7 ± 8.8[a]	19.2 ± 10.3[a]
CL_R (L/h)	9.4 ± 2.8	10.1 ±5.2	15.2 ± 5.5	11.7 ± 5.8

Data are represented as mean ± S.D. [a]: *p<0.05* vs. 25 mg; [b]: *p<0.05* vs. 100 mg; [c]: *<0.05* vs.300 mg by one-way *ANOVA* analysis. $AUC_{0\text{-inf}}$: area under the concentration-time curve from time 0 to infinity; $AUC_{0\text{-last}}$: area under the concentration-time curve from time 0 to last sampling time; C_{max}: maximum concentration observed in plasma; T_{max}: time to reach maximum concentration observed in plasma; $T_{1/2}$: terminal elimination half-life; CL/F: apparent clearance; VZ/F: apparent volume of distribution; $Ae_{0\text{-}24h}$: cumulative amount of unchanged drug excreted in urine from time zero to 24 h; $fe_{0\text{-}24h}$: fraction of unchanged drug excreted in urine from time zero to24h; CLR:renal clearance.

Table 3: Pharmacokinetic parameters of DBPR108 after once daily multiple oral administrations in healthy human male subjects.

Parameter		Dose Cohort		
	25 mg	**100 mg**	**300 mg**	**600 mg**
		Day 1		
$AUC_{0\text{-}24h}$ (ng*h/mL)	179 ± 41	1,050 ± 181[a]	2,620 ± 393[ab]	8,340 ± 1,070[abc]
$AUC_{0\text{-}inf}$ (ng*h/mL)	182 ± 41	1,090 ± 174[a]	2,840 ± 488[ab]	8,990 ± 1,200[abc]
C_{max} (ng/mL)	34 ± 14	141 ± 31	349 ± 88[ab]	1,100 ± 233[abc]
T_{max} (h)	3.17 ± 0.75	4.33 ± 1.37	3.17 ± 1.69	2.92 ± 1.28
$T_{1/2}$ (h)	2.28 ± 0.18	5.06 ± 0.75[a]	5.77 ± 0.99[a]	6.60 ± 0.90[ab]
CL/F (L/h)	142 ± 29	93 ± 15[a]	108 ± 20[a]	68 ± 10[abc]
Vz/F (L)	469 ± 108	689 ± 195	895 ± 180[a]	651 ± 159
$Ae_{0\text{-}24h}$ (mg)	1.4 ± 0.7	10.7 ± 2.1	36.2 ± 7.8[ab]	95.8 ± 14.0[abc]
$fe_{0\text{-}24h}$ (%)	5.7 ± 2.7	10.7 ± 2.1[a]	12.0 ± 2.6[a]	16.0 ± 2.3[abc]
CL_R (L/h)	8.0 ± 2.4	10.2 ± 0.6[a]	13.7 ± 1.7[ab]	11.5 ± 0.9[ac]
		Day 8		
$AUC_{0\text{-}24h}$ (ng*h/mL)	193 ± 46	1,170 ± 261[a]	2,820 ± 642[ab]	9,890 ± 1,210[abcd]
$AUC_{0\text{-}last}$ (ng*h/mL)	190 ± 47	1,350 ± 333	3,310 ± 960[ab]	11,400 ± 1,900[abc]
$AUC_{0\text{-}inf}$ (ng*h/mL)	206 ± 52	1,440 ± 358	3,440 ± 942[ab]	11,600 ± 2,130[abcd]
C_{max} (ng/mL)	36 ± 12	141 ± 29	372 ± 111[ab]	1,220 ± 330[abc]
T_{max} (h)	1.92 ± 0.97	2.25 ± 1.17	2.33 ± 0.75	3.17 ± 0.75
$T_{1/2}$ (h)	4.61 ± 3.12	12.20 ± 4.57[ad]	13.50 ± 6.45[ad]	10.30 ± 5.79
CL/F (L/h)	139 ± 46	88 ± 17[a]	111 ± 24	62 ± 8[ac]
Vz/F (L)	863 ± 505	1,530 ± 622	2,120 ± 1,070[ad]	888 ± 462[c]
$Ae_{0\text{-}24h}$ (mg)	1.4 ± 0.3	13.2 ± 2.3	42.0 ± 4.0[ab]	113.0 ± 30.9[abc]
$fe_{0\text{-}24h}$ (%)	5.6 ± 1.3	13.2 ± 2.3[a]	14.0 ± 3.1[a]	18.9 ± 5.1[abc]
$Ae_{0\text{-}48h}$ (mg)	1.5 ± 0.4	14.4 ± 2.5	47.9 ± 11.3[ab]	130.0 ± 34.4[abc]
$fe_{0\text{-}48h}$ (%)	5.8 ± 1.4	14.4 ± 2.5[a]	16.0 ± 3.8[a]	21.6 ± 5.7[abc]
CL_R (L/h)	7.4 ± 0.9	10.8 ± 1.3[a]	15.0 ± 2.0[ab]	11.6 ± 2.4[ac]
AR (%)	**110 ± 28**	**114 ± 27**	**107 ± 19**	**120 ± 18**

Data are represented as mean ± S.D. [a]: *p<0.05* vs. 25 mg; [b]: *p<0.05* vs. 100 mg; [c]: *p<0.05* vs. 300 mg; [d]: *p<0.05* vs. Day1 of the equal dose, by one-way *ANOVA* analysis following by *Student-Newman-Keuls* multiple comparison test. $AUC_{0\text{-}24h}$: area under the concentration-time curve from time 0 to 24 h; $AUC_{0\text{-}inf}$: area under the concentration-time curve from time 0 to infinity; $AUC_{0\text{-}last}$: area under the concentration time curve from time 0 to last sampling time; C_{max}: maximum concentration observed in plasma; T_{max}: time to reach maximum concentration observed in plasma; $T_{1/2}$: terminal elimination half-life; CL/F: apparent clearance; VZ/F: apparent volume of distribution; $Ae_{0\text{-}24h}$: cumulative amount of unchanged drug excreted in urine from time zero to 24 h; $fe_{0\text{-}24h}$: fraction of unchanged drug excreted in urine from time zero to 24 h; $Ae_{0\text{-}48h}$: cumulative amount of unchanged drug excreted in urine from time zero to 48 h; $fe_{0\text{-}48h}$: fraction of unchanged drug excreted in urine from time zero to 48 h; CL_R: renal clearance; AR:accumulation ratio.

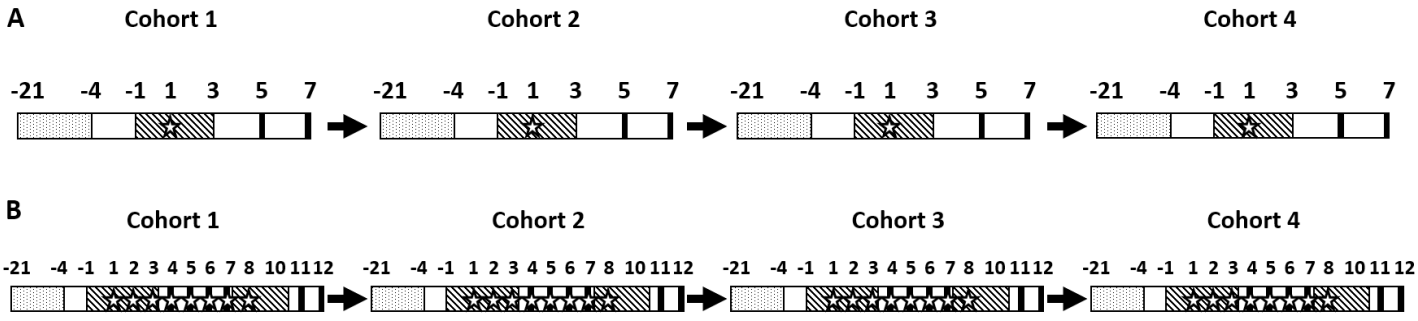


Figure 1. Study design of Phase I study of DBPR108.

The Phase 1 study of orally ingested DBPR108 is consisted of a single-dose (Figure 1A) and a follow-up multiple-dose (Figure 1B) study phases. Cohorts 1-4 were healthy male volunteers given oral doses of 25, 100, 300, and 600 mg, respectively, to which each cohort consisted of 6 DBPR108-treated and 2 placebo-treated subjects double-blinded and randomized. Study activities including pre-enrollment screening period from Day -21 to Day -4 (⁞), hospital visits (▌), hospital residential stays (▨) and oral ingestions (☆) of the Phase 1 study are indicated. The oral ingestion of DBPR108 was started from the lowest single dose level at 25 mg and the safety and tolerability were evaluated and confirmed before going up to the next higher single dose level gradually up to the highest dose level at 600 mg (Figure 1A). Analogously, the same study design, study activities and safety confirmations for the dose increments between cohorts were applied to the multiple-dose study phase, in which DBPR108 was ingested once daily by the healthy male volunteers from Day 1 to Day 8 (Figure 1B).

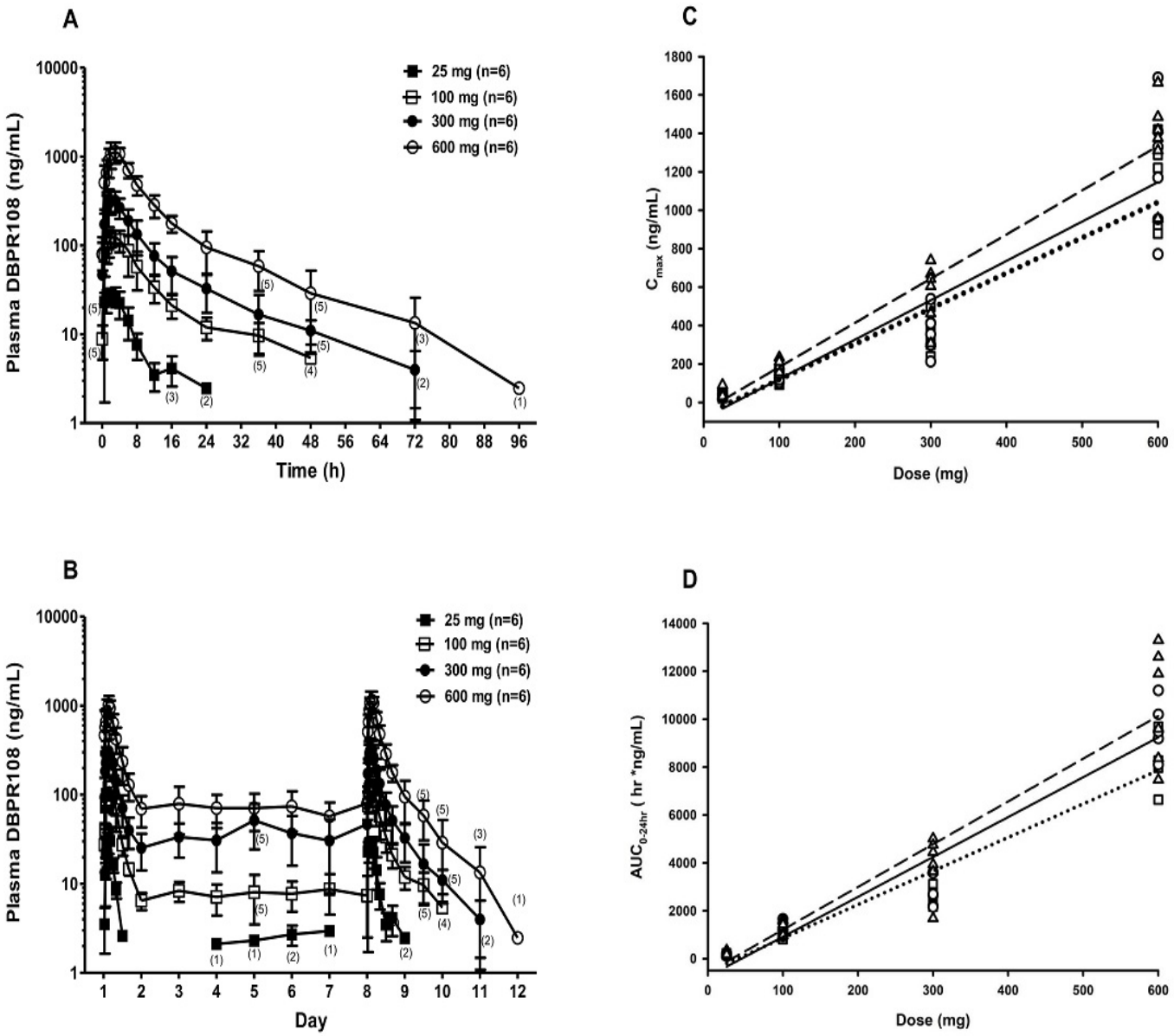
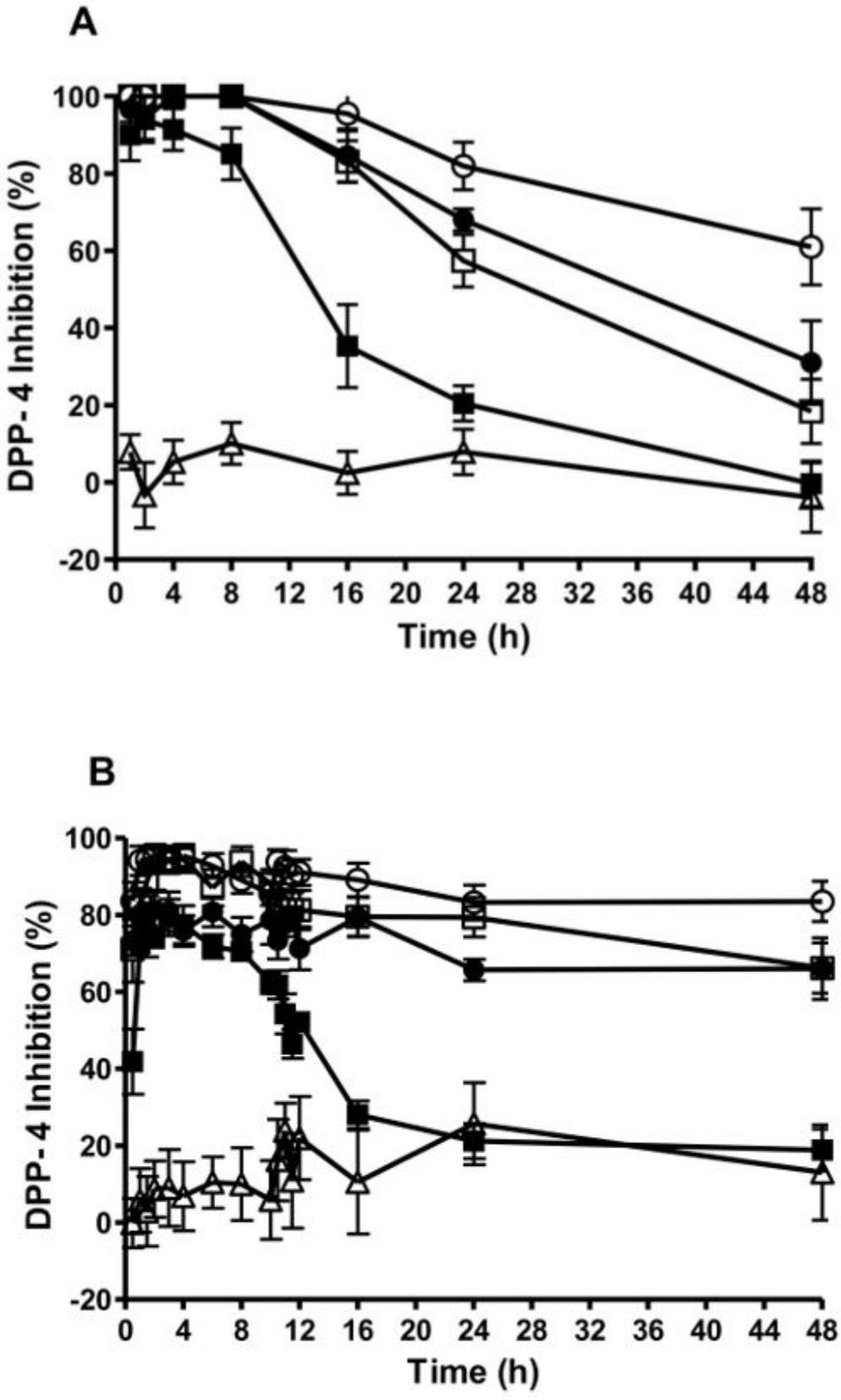


Figure 2. Mean plasma concentration-time profiles and pharmacokinetic linearity of the oral doses of DBPR108 in healthy male subjects.

Healthy male volunteers received one single oral dose (Figure 2A) and repeated once daily oral doses for 8 consecutive days (Figure 2B) of DBPR108 at different dose levels as 25 mg (■, N=6), 100 mg (□, N=6), 300 mg (●, N=6) and 600 mg (○, N=6). It is noted that the plasma DBPR108 concentrations were able to be maintained in a dose dependent manner during the daily visits (Days 4 to 7) between the 2 hospital stays. The number in the parentheses means the number of data available and presented. Data are presented as mean ± S.D. The C_{max} (Figure 2C) and AUC_{0-t} (Figure 2D) of plasma DBPR108 collected from all the healthy male volunteers were plotted against the 4 oral dose levels in both single- and multiple-dose. Values of the three data sets of C_{max} and AUC_{0-t} from the single-dose study (△, --- dash line, N=24), and from the multiple-dose study on Day 1 (□, … dotted line, N=24) and on Day 8 after repeated once daily oral doses for 8 consecutive days (○, — solid line, N=24) were analyzed and shown. C_{max} and AUC_{0-t} were increased with doses in positive proportion as presented by the three linear regression lines.


A
DPP-4 Inhibition (%)
100
80
60
40
20
0
-20
0 4 8 12 16 20 24 28 32 36 40 44 48
Time (h)
B
DPP-4 Inhibition (%)
100
80
60
40
20
0
-20
0 4 8 12 16 20 24 28 32 36 40 44 48
Time (h)

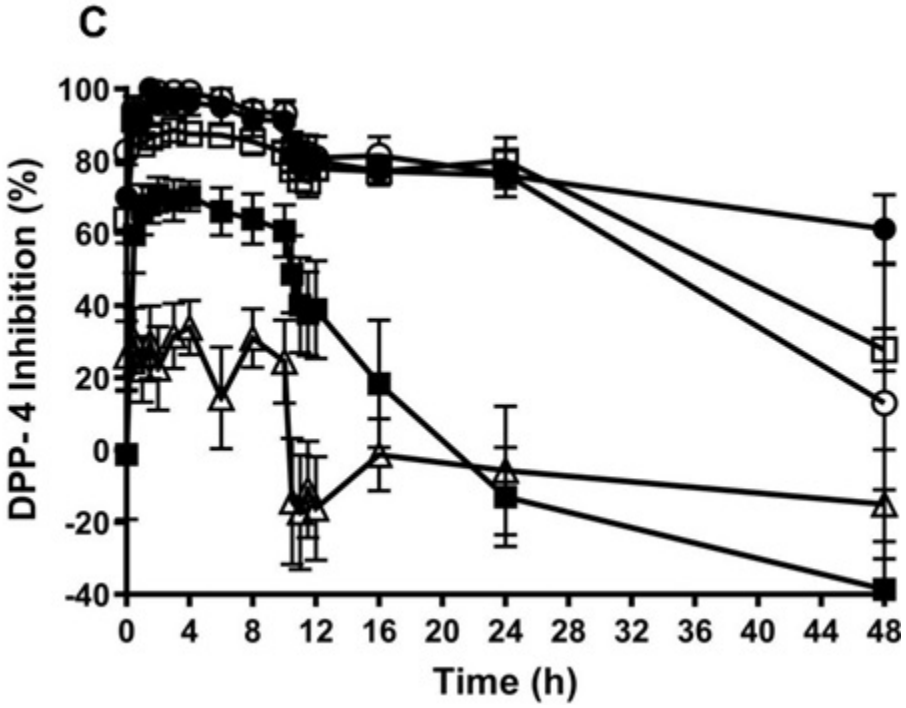


Figure 3. Inhibition of plasma DPP-4 activities after oral administrations of DBPR108 in healthy male subjects.

Healthy male volunteers received single dose (Figure 3A) or repeated doses (Day 1, Figure 3B; Day 8,Figure 3C) of DBPR108 via oral ingestion at different dose levels: 0 mg, (Placebo, △, N=8), 25 mg (■, N=6), 100 mg (□, N=6), 300 mg (●, N=6) and 600 mg (○, N=6). Within a time period from pre-dose to 48 h after an oral ingestion, plasma samples were collected at the time points indicated for measurements of the DPP-4 activities. A significant dose-dependent inhibitory effect of DBPR108 on the plasma DPP-4 activity was observed on both Day 1 after one single dose administered and Day 1 and Day 8 after the administrations of repeated oral doses. Data normalized in percentage to the maximum inhibition of the plasma DPP-4 activity are presented as mean ± S.D.

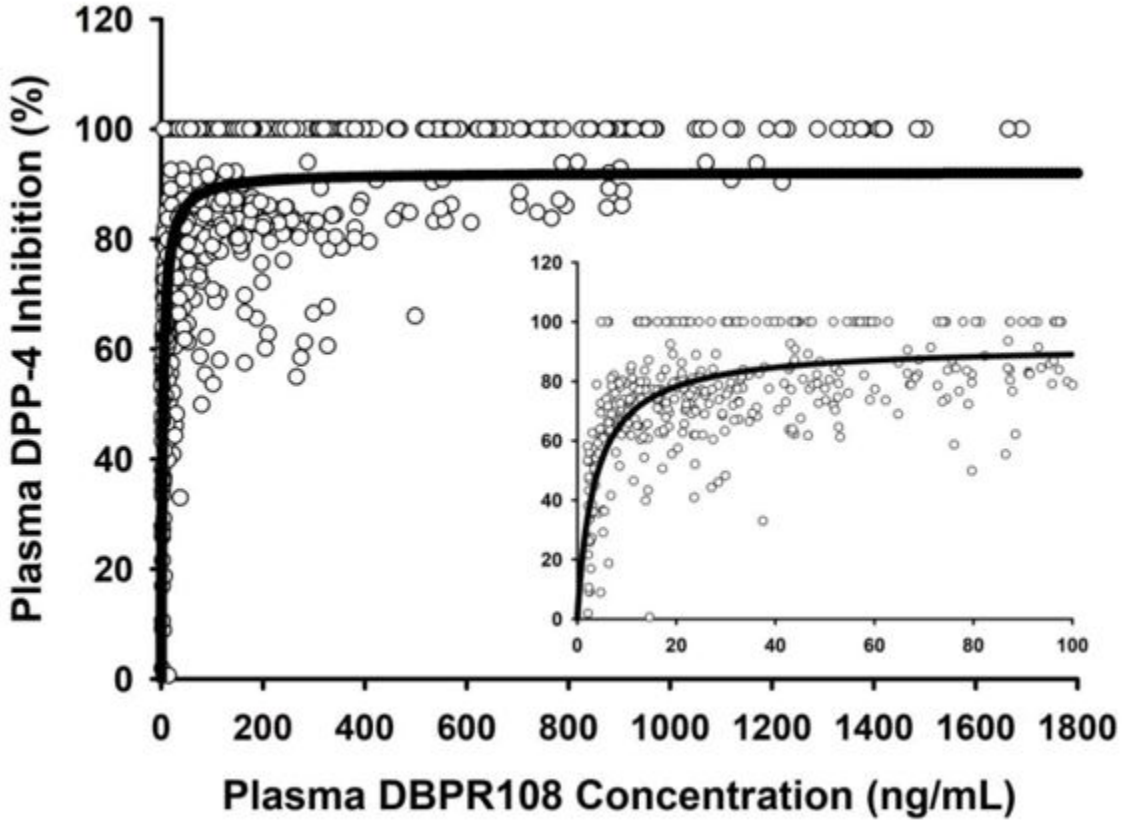


Figure 4. Correlation between the plasma concentrations of DBPR108 and the corresponding percent inhibitions of plasma DPP-4 activity in healthy male subjects.

All data matched in pairs of both measurable values of plasma DPP-4 activity inhibition in percentage and plasma DBPR108 concentrations in all of the healthy male volunteers were included. A non-linear regression analysis was performed to demonstrate the good concentration-dependent correlation between these two parameters of interest. An insert is included to better express the data and regression line for the DBPR108 concentrations at ≦100 ng/mL.

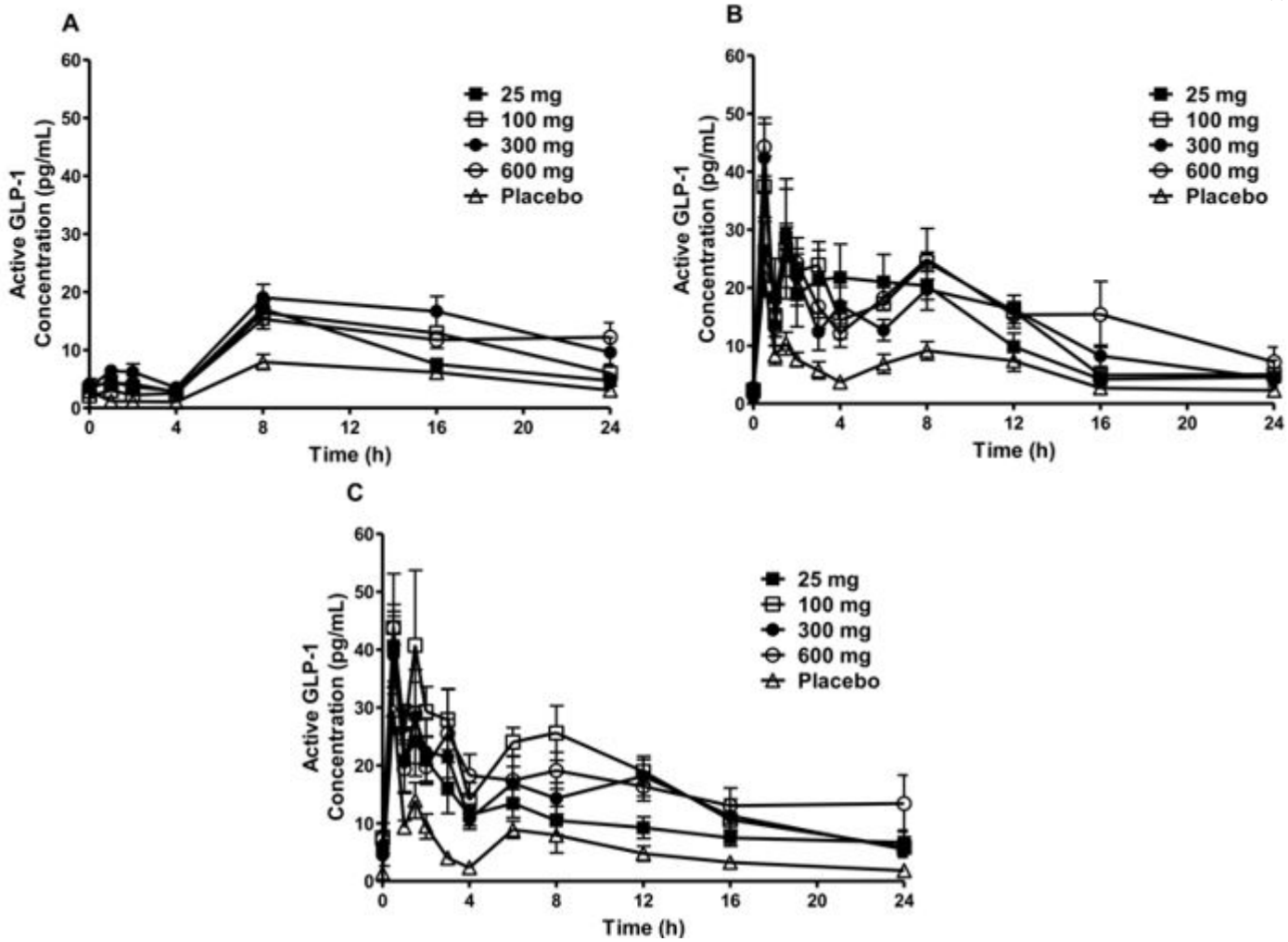


Figure 5. Increase of the circulating active form of GLP-1 after oral administration of DBPR108 in healthy male subjects.

Healthy male volunteers received oral dose(s) of DBPR108 at different dose levels; 0 mg, (Placebo, △, N=8), 25 mg (■, N=6), 100 mg (□, N=6), 300 mg (●, N=6) and 600 mg (○, N=6). The circulating levels of GLP-1 of active form were increased in response to the DBPR108 administration in the single-dose study (Figure 5A), and in the multiple-dose study on Day 1 (Figure 5B) and Day 8 (Figure 5C). The subjects in the single-dose study were fasted with restriction of food and water for at least 10 h before the DBPR108 dosing and for another fasting period of 4 h after the DBPR108 dosing. On the other hand, the subjects in the multiple-dose study were non-fasted and served with a total of 4 standardized test meals as breakfasts and dinners on the 2 dosing days, Day 1 and Day 8, during the 2 hospital stays. Breakfast test meals were given approximately 5 min after the DBPR108 dosing. A significant dose-dependent induction activity of DBPR108 on the circulating active GLP-1 level was observed in both study phases. Data are presented as mean ± S.D.

4.3. Pharmacodynamic Profiles Measured

DBPR108 of 25 to 600 mg orally given to the healthy male subjects in one single dose rapidly inhibited the circulating plasma DPP-4 activity in a dose dependent manner. The inhibited activity was expressed in percentage of DPP-4 inhibition after being normalized to the maximum inhibitory effect (E_{max}) and calculated as [100*(baseline activity-measured value)/baseline activity] for a better comparison of the DPP-4 data. The baseline activity was defined as the measured DPP-4 activity value at predose or time of 0 h (Figure 3A). The maximum inhibitory activities (E_{max}s) at 100% were achieved at 1 to 4 h and plasma DPP-4 activity was completely suppressed in all subjects for up to 8 h in cohorts given in a dose of 100 mg or greater. During this time period, the plasma DPP-4 activities in all DBPR108-treated cohorts were essentially completely suppressed and therefore the E_{max}s of all dose cohorts were not different from each other. At the dose level of 100 mg or greater, the plasma DPP-4 activity suppressed did not return to the baseline by 48 h after the DBPR108 dosing. In multiple-dose phase study, administrations of DBPR108 at multiple oral doses of 25 to 600 mg dose dependently inhibited the plasma DPP-4 activities and the inhibition profiles on Day 1 and Day 8 were shown in Figure 3B and Figure 3C, respectively. The maximum inhibition on plasma DPP-4 activities were quickly reached at 0.5 to 1 h and last till 2 to 8 h after the DBPR108 dosing. At a dose of 100 mg or greater, there was an approximately 70% or higher inhibition on plasma DPP-4 activity over the 24-h after dosing period on Day 1 and Day 8. It was noticed that the percentages of inhibition on the plasma DPP-4 activity were in positive and good correlation (R = 0.8579) with the concentrations of DBPR108 in the systemic circulation of the study subjects in both single- and multiple-dose studies (Figure 4). DBPR108 increased the circulating levels of active

GLP-1 significantly higher than that of placebo in almost all subjects of DBPR108-treated groups in both the single- and multiple-dose studies (Figure 5). Nonetheless, no further significant increase in the circulating active GLP-1 levels was found among dose groups of 25 mg up to 600 mg. Noted in Figure 5A, marginal basal levels of circulating active GLP-1 were measured during the 4-h fasting period after the DBPR108 dosing in the morning in the single-dose study. A clear increase of the circulating plasma active GLP-1 was observed after the end of the 4-h fasting period, on which a lunch (given at 4 h after DBPR108 dosing) and later a dinner (given at 10 h after DBPR108 dosing) were given to the study subjects (Figure 5A). On the other hand, a non-fasting approach, with breakfast, lunch, and dinner, was designed in the multiple-dose study and therefore, the circulating active GLP-1 levels increased upon breakfast intake in the study subjects of all dose groups including placebo in the morning on Day 1 (Figure 5B) and Day 8 (Figure 5C). A significant surge of the circulating active GLP-1 level in response to the breakfast ingestion in the non-fasting subjects (Figure 5B and 5C) was detected differently from those fasting ones (Figure 5A) within the 4-h interval immediately after drug administration, 5-min later, accompanied by the breakfast test meal intake. However, a similar concentration profile of the circulating active GLP-1 was observed during the 4 to 24 h time interval after DBPR108 dosing in both single- and multiple-dose studies. By calculating the areas under the circulating active GLP-1 levels changed from the baseline as exposure of active GLP-1 for comparison, they were estimated for DBPR108 at 25, 100, 300 and 600 mg as a degree of exposure of 3-, 7-, 9-, 8-fold that of placebo, respectively. Similarly, in the multiple-dose phase study, an exposure for 25, 100, 300, and 600 mg DBPR108 was approximately 3-, 4-, 4-, and 5-fold that of placebo on Day 1 and approximately 3-, 5-, 5-, and 5-fold that of placebo on Day 8, respectively. The pharmacokinetic and pharmacodynamic study results of both single-dose (fasting) and multiple-dose (non-fasting) studies showed similar pharmacokinetic parameters (Figure 2, Tables 2 and 3), plasma DPP-4 inhibition (Figure 3) and increase of circulating active GLP-1 levels (Figure 5), indicating that an effect of food intake affecting the oral absorption and of DBPR108 in healthy humans is considered dismal. DBPR108 did not affect blood glucose and no blood glucose levels were found below the low normal range limit of 75 mg/dL after the oral doses of DBPR108 in both phase studies (Supplemental Materials). Furthermore, DBPR108 did not significantly affect the circulating level and systemic exposure of glucagon, total GLP-1, insulin, and C-peptide in all single- and multiple-dose cohorts (Supplemental Materials).

4.4. Acceptable Tolerability Assessed

In the single-dose study, there were no deaths, severe adverse effects (SAEs), adverse events of special interest (AESIs), or discontinuations from the study due to adverse effects following a single oral dose of 25, 100, 300, or 600 mg DBPR108 to the healthy adult Asian male subjects. There were no clinically meaningful changes or trends noted in clinical laboratory tests, vital signs, or ECG findings and physical examinations. No safety or tolerability concerns were identified in the participated subjects in the single-dose study in which DBPR108 was well tolerated with no episodes of hypoglycemia or pancreatitis. There were 4 reported treatment emergent adverse effects (TEAEs) in 3 of the 24 (12.5%) DBPR108-treated subjects. They were decreased platelet count, hematuria, minor infection and ligament sprain and all these 4 TEAEs resolved before the end of the study with an unknown relationship to the study drug DBPR108. The decrease in platelet count was observed beginning on Day 3 (28-year-old, 300 mg DBPR108) from a platelet count of 152 $\times 10^3/\mu L$ (normal range: 130-400 $\times 10^3/\mu L$) at the time entered the study down to 145 $\times 10^3/\mu L$ on Day 3 and the event improved on Day 5 for a follow-up visit at 166 $\times 10^3/\mu L$, for which the event was considered resolved. Hematuria and minor infection were diagnosed on Day 3 (24-year-old, 600 mg DBPR108) with urinalysis findings of 2-5 erythrocytes per high-power field, but otherwise normal and, on Day 5 at the follow-up visit, urinary erythrocytes of 0-2 cells per high-power field. Both events resolved on Day 5 at the follow-up visit. Ligament sprain experienced beginning on Day 4 (24-year-old, 600 mg DBPR108) and resolved on Day 7 for the end-of-study visit. Therefore, all these 4 TEAEs were resolved before the end of the study with an unknown relationship to the study drug in the single-dose study. In the multiple-dose study, there were no safety or tolerability concerns identified following 8 consecutive once daily oral doses of 25, 100, 300, or 600 mg DBPR108 to the healthy subjects. There were no clinically significant changes or trends noted in clinical laboratory tests, vital signs, ECG findings, and physical examinations. DBPR108 was well tolerated with no episodes of hypoglycemia or pancreatitis and no other episode directly attributed by DBPR108. There were 2 reported TEAEs in 2 of the 24 (8.3%) DBPR108-treated subjects, including atrioventricular block first degree and dry mouth. A case of atrioventricular block first degree was observed at approximately 1 h after dosing on Day 8 (31-year-old, 300 mg DBPR108), which resolved approximately 2 h later. A TEAE of dry mouth was observed after dosing on Day 1 to Day 5 (31-year-old, 600 mg DBPR108), which resolved later on Day 5. Both TEAEs were mild in intensity and with possible relationships to the treatments, and resolved before the end of this multiple-dose study.

5. Discussion

DBPR108 showed quick (T_{max}=1.8 h) oral absorption in animals [27] and no dose-limited oral absorption up to 2000 mg/Kg in rats or 1000 mg/Kg in dogs and monkeys. In agreement, the present first-in-human study reported that DBPR108 was quickly absorbed after oral doses of 25-600 mg in healthy Asian male adults. Rapid oral absorption (T_{max}=1.5-4 h) of DBPR108 was observed with a linear pharmacokinetic profile as demonstrated by the dose-dependent relationships both in C_{max} and systemic exposure AUC (Figure 2) within the dose range of 25-600 mg/day, in which effective and prolonged inhibition of plasma DPP-4 activity was observed and, therefore, there is no need to explore its pharmacokinetic character-

istics at higher doses in humans. Whether the low fraction (<20%) of unchanged DBPR108 excreted in urine provides a good safety profile of less kidney-associated adverse effects remains to be investigated to see if there is a need to adjust its therapeutic doses in renal functions impaired patients. Lack of a food effect on the oral absorption and pharmacological efficacies of DBPR108 provides advantages in clinical uses. The trough plasma DBPR108 concentrations in between the 2 hospital stays were detectible and these trough concentrations were able to maintain in a dose-dependent manner during the daily visits in which the once daily oral doses were administered (Figure 2B). Meanwhile, there were no significant changes in the DBPR108 trough concentrations of the 100-600 mg-treated cohorts, indicating a steady state readily achieved after the once daily oral doses in the range of 100-600 mg. Overall, to treat patients with DBPR108 at once daily oral doses of 25-600 mg/day is feasible and optimal efficacious dose levels are to be explored further in clinical studies for treating T2DM patients.

It was noted that the DBPR108-inhibited plasma DPP-4 activities measured were well-correlated positively with the plasma concentrations of DBPR108 attained (Figure 4) in the systemic circulation across all dose levels. The more DBPR108 molecules presented in the plasma, the higher percentages inhibiting the plasma DPP-4 activity were achieved in a non-linear hyperbolic relationship regardless whether the data were from the single- or multiple-dose studies. While DBPR108 inhibited plasma DPP-4 activities and increased circulating active GLP-1 levels, it did not significantly affect plasma glucose, glucagon, total GLP-1, insulin, and C-peptide exposure in the circulation in both single- and multiple-dose studies. The reason for the lack of a significant change in glucose and insulin levels in the current study is unknown. Nevertheless, lack of an increase of insulin level in the fasting healthy male subjects could be explained in part by the findings that intravenous infusion of GLP-1 induced only marginal changes in the levels of insulin and C-peptide and concluded that GLP-1 could not increase the circulating insulin to a level that causes hypoglycemia in healthy subjects who have normal fasting glucose concentrations [28]. Reported previously, DBPR108 effectively inhibited plasma DPP-4 activity in *db*/*db* mice, rats, dogs, and cynomolgus monkeys as well as increased circulating active GLP-1 levels and tolerability, together with metformin, to oral blood glucose challenge in animals [27]. In agreement with these preclinical findings, DBPR108 dose-dependently inhibited plasma DPP-4 activity and increased the circulating level of active GLP-1 compared to that of placebo in a dose range of 25-600 mg in the healthy male subjects. Food intake, though caused an increase and disturbance on the circulating GLP-1 levels across all dose cohorts (Figures 5B and 5C), is again not to affect the inhibition profiles on plasma DPP-4 activity by orally ingested DBPR108 (Figures 3B and 3C). The quick onset of plasma DPP-4 inhibition, lengthy (up to 8 h) complete inhibition, and sustained plasma DPP-4 activity suppression for up to 24 h over the dose range of 100-600 mg DBPR108 suggest that a regimen of once daily multiple oral doses of DBPR108 at ≧100 mg for clinical uses in patients is feasible. Furthermore, DPP-4 inhibitors provide clinical benefits of reduced HbA1c, lower risk of hypoglycemia and weight loss when used in combination with metformin for T2DM therapy [29,30] and combination uses of DBPR108 with metformin also demonstrated an increase of oral glucose tolerability in diabetic diet-induced obesity mice [27]. These preclinical and clinical findings support the combination use of DBPR108 with metformin for treating T2DM patients. In general, currently available DPP-4 inhibitor drugs show significant antidiabetic effects and are generally safe and well tolerated. However, there are still (severe) adverse events occurred, such as infections, prostate cancer, inflammatory bowel disease, anaphylactoid reactions, angioedema, exfoliate dermatologic reactions, bile duct stone, acute pancreatitis and severe joint pain [21-25]. Increased risks for saxagliptin and alogliptin with heart failure, particularly in patients who already have heart or kidney diseases [31-33], for sitagliptin and vildagliptin with nasopharyngitis, urinary tract infection and frequency of headache [34], and for taking DPP-4 inhibitors, compared to metformin, with acute kidney injury [35] had been observed. Omarigliptin and trelagliptin were developed for a once-weekly dosing convenience and better patient satisfaction and compliance. Nevertheless, adverse effects were reported such as nasopharyngitis, prostate cancer and bile duct stone (patient discontinued) for omarigliptin [24] and acute pancreatitis (1 out 14 subjects) with increased lipase and several other TEAEs for trelagliptin [25]. Both omarigliptin and trelagliptin were not applying for marketing approvals from the US-FDA. DBPR108 presenting a novel chemical structure distinct from all of the other available DPP-4 inhibitors, with potent DPP-4 enzyme inhibition, linear pharmacokinetic properties for an ease of dose adjustment, relatively less accumulated amount excreted via the renal route, and promising safety and tolerability profiles, shall provide a safe and satisfied clinical therapeutic outcomes in T2DM patients. DBPR108 was orally gavaged at 100-2000 mg/Kg/day for 14 days in rats and showed no clinical gross behavior signs across all doses. The non-observed-adverse-effect-level (NOAEL) was estimated at a high dose of 1000 mg/Kg/day in rats and dogs in a 28-day toxicology study [27]. No adverse effects of skin lesion or gross/microscopic changes were observed in DBPR108-treated cynomolgus monkeys at 200 mg/Kg/day after a once daily oral treatment period of 13 consecutive weeks (unpublished results). Furthermore, DBPR108 exhibited advantages in its high specificity of inhibition on DPP-4 against the other prolyl-cleaving proteases family members with an IC_{50} of 15 nM, >100 μM, >100 μM, and >100 μM on human DPP-4, DPP-8, DPP-2, and DPP-9, respectively [26]. Compared in parallel, sitagliptin and vildagliptin showed an IC_{50} of 23 nM, >100 μM, >100 μM, and >100 μM for sitagliptin and 56 nM, 18.5 μM, >50 μM, and 1.2 μM for vildagliptin against human DPP-4, DPP-8, DPP-2, and DPP-9, respectively (unpublished results). Therefore, a safe or less-side-effect profile for DBPR108 may be expected in human uses, comparable to sitagliptin or better than vildagliptin regarding safety or tolerability. DBPR108 does not inhibit other unrelated proteases and ion channels exam-

ined in the safety pharmacology profiling assays (data not shown). DBPR108, in good safety profiles, entered this first-in-human study and showed no concerns of safety or tolerability across the 8 consecutive daily oral doses of 25-600 mg to the healthy, adult Asian males participated. Furthermore, there were no episodes of hypoglycemia, pancreatitis, or any others directly attributed by DBPR108 observed in all dose cohorts. Overall, with a linear pharmacokinetic profile and an 8-h long close to complete plasma DPP-4 inhibition in a dose of ≧100 mg/day, DBPR108 may be given once daily orally in T2DM patients including those with renal functions impaired. Optimal oral dose levels of DBPR108 at <600 mg/day and uses in combination with metformin are to be decided for further clinical studies.

More than just an antidiabetic drug target, DPP-4 enzyme has been investigated broadly and there are several substrates other than incretins GLP-1 and glucose-dependent insulinotropic polypeptide such as stromal cell-derived factor-1, neuropeptide Y, polypeptide YY, atrial natriuretic peptide, brain natriuretic peptide, oxyntomodulin, and pituitary adenylate cyclase-activating polypeptide [36,37]. Therefore, significant biological effects of DPP-4 other than the systemic glycemic control via incretins regulation may be expected. DPP-4 was interacting with integrin beta-1 and involving tumor growth factor-beta signaling pathways such that linagliptin treatment attenuated the profibrotic endothelial-to-mesenchymal transition in mice kidney [38]. However, a recent randomized placebo-controlled trial failed to demonstrate benefits of linagliptin for protecting the T2DM patients at high risk of cardiovascular and kidney events [39]. Interestingly, DPP-4 is associated with autoimmune disease and its enzymatic activity was increased in the saliva of patients with Sjögren's Syndrome [40]. DPP-4 inhibitors improved liver injury and reduced liver fat in patients of non-alcoholic fatty liver disease [41] and decreased mortality of all causes, independent of glucose control, after long term treatment in the veteran population [42]. Benefits of less beta-amyloid amount in the brains and longitudinal cognitive outcome were observed in diabetic patients of Alzheimer's disease-related cognitive impairment [43]. Recently, DPP-4 inhibitors offered better clinical outcomes and decreased mortality in T2DM patients PCR-tested positive for COVID-19 [44,45] although a call for randomized controlled trials was suggested to see if DPP-4 inhibitors have clinical benefits for COVID-19 therapy [46]. It will be not surprised to see more novel preclinical and clinical findings in which DPP-4 inhibition is involved in the future.

6. Conclusion

The present first-in-human double-blind, randomized, placebo-controlled Phase I study reported that DBPR108 is readily absorbable after oral administrations at different doses with minimum accumulation in repeated once daily doses and inhibits the plasma DPP-4 activity and increases circulating active GLP-1levels in the healthy adult Asian male subjects. DBPR108 has no safety or tolerability concerns after given multiple oral doses up to 600 mg/day with no episodes of hypoglycemia or pancreatitis observed. These promising pharmacokinetic, pharmacodynamic, and tolerability study results suggest a feasibility and good safety of once daily repeated oral doses, given with or without food, up to 600 mg of DBPR108 for further investigations in clinical uses treating T2DM patients, possibly including those with renal functions impaired and under monitored. In addition, clinical studies for DBPR108 used alone or in combination with others such as metformin as T2DM therapeutics are warranted.

7. Key Findings

DBPR108 was orally absorbable, without food effect, exhibiting maximum plasma DBPR108 concentrations (Cmax) of 34-1370 ng/mL, time to reach Cmax (Tmax) at 1.9-4.3 h, and elimination half-life of 2.3-18.6 h across all doses. Unchanged DBPR108 was recovered at 5.7-21.6% of the oral dose in urine. Similar to Cmax, systemic exposure AUC of DBPR108 was increased dose-dependently and minimum accumulation of DBPR108 was observed after 8 repeated daily doses across all cohorts. DBPR108 significantly inhibited plasma DPP-4 activity and increased circulating active GLP-1 levels. Without subject discontinuation, there were no significant dose-dependent trends in vital signs, clinical laboratory tests, electrocardiograms, and adverse events such as hypoglycemia or pancreatitis observed throughout the study.

8. Acknowledgments

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Supplimentary Materials

Supplemental Table 1: Mean change-from-baseline plasma glucose in single-dose phase study.

Parameters	Placebo	Dose Cohort			
		25mg	100mg	300mg	600mg
AUC0-48h (mmol.h/L)	21.4 ± 14.3	23.1 ± 8.1	22.7 ± 7.6	3.9 ± 9.3	15.1 ± 3.6
E_{max} (mmol/L)	1.27 ± 0.53	1.38 ± 0.47	1.11 ± 0.37	0.51 ± 0.30	0.95 ± 0.22
T_{Emax} (H)	12.03	8.03	16.05	12.03	16.02
	(8.02, 16.03)	(8.02, 16.03)	(8.07, 24.05)	(8.02, 24.03)	(8.02, 48.07)

Data are expressed as mean ± S.D.

TEmax is presented as median (minimum, maximum).

AUC0-48h: area under the response versus time curve from time zero to 48 h were calculated using the change-from-baseline data; Emax: maximum concentration observed in plasma; TEmax: time to reach maximum concentration observed in plasma.

Supplemental Table 2. Mean change-from-baseline plasma glucagon in single-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
AUC0-48h (mmol.h/L)	-11.2 ± 123	82.6 ± 80.4	6.8 ± 117	32.1 ± 114	23 ± 145
Emax (mmol/L)	4 ± 3	6 ± 3	4 ± 2	5 ± 3	4 ± 4
TEmax (h)	16.03	12.03	20.03	16.03	12.03
	(1.03, 48.07)	(8.02, 48.05)	(2.03, 48.07)	(16.03, 48.07)	(8.02, 48.03)

Data are expressed as mean ± S.D.

TEmax is presented as median (minimum, maximum).

AUC0-48h: area under the response versus time curve from time zero to 48 h were calculated using the change-from-baseline data; Emax: maximum concentration observed in plasma; TEmax: time to reach maximum concentration observed in plasma.

Supplemental Table 3. Mean change-from-baseline plasma total glucagon-like peptide-1 in single-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
AUC0-48h (mmol.h/L)	114 ± 105	128 ± 129	187 ± 171	359 ± 208	181 ± 109
Emax (mmol/L)	10.9 ± 7.3	10.5 ± 3.7	13.6 ± 6.9	19.1 ± 9.2	13.0 ± 5.1
TEmax (h)	8.03	8.02	8.05	16.03	24.03
	(8.02, 16.03)	(8.02, 8.03)	(8.03, 16.03)	(8.03, 16.03)	(8.03, 48.03)

Data are expressed as mean ± S.D.

TEmax is presented as median (minimum, maximum).

AUC0-48h: area under the response versus time curve from time zero to 48 h were calculated using the change-from-baseline data; Emax: maximum concentration observed in plasma; TEmax: time to reach maximum concentration observed in plasma.

Supplemental Table 4. Mean change-from-baseline serum insulin in single-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
AUC0-48h (mmol.h/L)	1.68 ± 2.16	1.29 ± 1.12	1.80 ± 1.27	0.71 ± 0.58	1.04 ± 0.62
Emax (mmol/L)	0.18 ± 0.20	0.17 ± 0.16	0.15 ± 0.10	0.13 ± 0.09	0.09 ± 0.06
TEmax (h)	16.02	8.02	16.03	12.03	16.02
	(8.02, 16.03)	(8.02, 8.03)	(8.03, 16.03)	(8.02, 48.05)	(8.02, 28.07)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}48h}$: area under the response versus time curve from time zero to 48 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in serum; T_{Emax}: time to reach maximum concentration observed in serum.

Supplemental Table 5. Mean change-from-baseline serum C-peptide in single-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
AUC0-48h (mmol.h/L)	69.6 ± 34.5	66.4 ± 29.1	64.9 ± 24.4	42.7 ± 25.6	45.1 ± 20.3
Emax (mmol/L)	5.7 ± 2.6	7.1 ± 4.0	4.9 ± 1.8	4.5 ± 1.7	3.5 ± 1.5
TEmax (h)	12.03	8.03	16.03	8.04	16.02
	(8.02, 16.03)	(8.02, 16.03)	(8.03, 16.07)	(8.02, 16.03)	(8.02, 16.05)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}48h}$: area under the response versus time curve from time zero to 48 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in serum; T_{Emax}: time to reach maximum concentration observed in serum.

Supplemental Table 6. Mean change-from-baseline plasma glucose in multiple- dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
Day 1					
AUC0-24h (mmol.h/L)	282 ± 167	287 ± 226	246 ± 90.6	250 ± 124	232 ± 156
Emax (mmol/L)	53.3 ± 22.8	37.2 ± 11.9	32.5 ± 10.2	38.5 ± 11.2	40.2 ± 8.0
TEmax (h) 11.00	11.0	10.5	10.5	11.0	5.5
	(0.50, 12.00)	(0.50, 11.50)	(0.50, 12.00)	(0.50, 12.00)	(0.50, 11.50)
Day 8					
AUC0-24h (mmol.h/L)	371 ± 187	321 ± 124	287 ± 76.6	421 ± 97.5	200 ± 86.8
Emax (mmol/L)	57.8 ± 28.6	40.5 ± 10.8	41.3 ± 12.7	54.0 ± 6.9	44.3 ± 12.5
TEmax (h)	10.75	10.5	10.75	10.75	8.25
	(6.00, 12.00)	(10.50, 11.50)	(10.50, 12.00)	(10.50, 11.50)	(0.50, 11.50)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}24h}$: area under the response versus time curve from time zero to 24 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in plasma; T_{Emax}: time to reach maximum concentration observed in plasma.

Supplemental Table 7. Mean change-from-baseline plasma glucagon in multiple- dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
Day 1					
AUC0-24h (mmol.h/L)	180 ± 460	226 ± 641	-241 ± 320	-290 ± 393	-85 ± 317
Emax (mmol/L)	44.7 ± 23.9	91.6 ± 94.4	27.9 ± 13.8	28.6 ± 15.9	33.6 ± 8.3
TEmax (h) 11.00	1.75	9.75	2.5	1.75	3.5
	(0.50, 10.50)	(0.50, 23.97)	(0.50, 3.00)	(0.50, 23.97)	(2.00, 10.50)
Day 8					
AUC0-24h (mmol.h/L)	219 ± 565	236 ± 582	-162 ± 419	-309 ± 426	-120 ± 227
Emax (mmol/L)	62.2 ± 39.6	47.0 ± 34.5	33.0 ± 17.7	23.5 ± 15.9	28.9 ± 17.5
TEmax (h)	0.5	1.5	1.5	1.5	2.25
	(0.00, 10.50)	(0.50, 24.00)	(0.50, 10.50)	(0.50, 16.00)	(0.50, 16.00)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}24h}$: area under the response versus time curve from time zero to 24 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in plasma; T_{Emax}: time to reach maximum concentration observed in plasma.

Supplemental Table 8. Mean change-from-baseline serum total glucagon-like peptide-1 in multiple-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
Day 1					
AUC0-24h (mmol.h/L)	-123 ± 464	-20.0 ± 263	-396 ± 349	-471 ± 309	-321 ± 355
Emax (mmol/L)	66.3 ± 32.1	44.4 ± 11.4	39.2 ± 22.1	47.5 ± 30.1	55.7 ± 21.0
TEmax (h) 11.00	0.5	1.0	0.5	0.5	0.5
	(0.50, 6.00)	(0.50, 16.00)	(0.50, 23.97)	(0.50, 2.00)	(0.50, 4.00)
Day 8					
AUC0-24h (mmol.h/L)	-370 ± 656	-199 ± 585	-691 ± 518	-745 ± 515	-696 ± 282
Emax (mmol/L)	71.1 ± 57.8	41.0 ± 34.2	39.8 ± 19.4	30.7 ± 25.6	50.7 ± 26.9
TEmax (h)	0.5	0.51	1.0	1.25	5.5
	(0.50, 1.50)	(0.50, 2.00)	(0.50, 24.00)	(0.50, 24.00)	(0.50, 10.50)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}24h}$: area under the response versus time curve from time zero to 24 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in serum; T_{Emax}: time to reach maximum concentration observed in serum.

Supplemental Table 9. Mean change-from-baseline serum insulin in multiple-dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
Day 1					
AUC0-24h (mmol.h/L)	498 ± 259	639 ± 181	445 ± 165	500 ± 96.8	-321 ± 355
Emax (mmol/L)	103 ± 68.0	94.2 ± 31.1	101 ± 39.2	129 ± 49.0	55.7 ± 21.0
TEmax (h) 11.00	0.75	3.5	0.5	0.5	0.5
	(0.50, 11.00)	(1.00, 11.00)	(0.50, 10.50)	(0.50, 11.00)	(0.50, 4.00)
Day 8					
AUC0-24h (mmol.h/L)	487 ± 214	643 ± 175	398 ± 121	594 ± 211	-696 ± 282
Emax (mmol/L)	90.4 ± 29.6	89.8 ± 39.8	76.6 ± 39.8	117 ± 30.8	50.7 ± 26.9
TEmax (h)	5.75	10.75	0.75	0.5	5.5
	(0.50, 12.00)	(0.50, 12.00)	(0.50, 12.00)	(0.50, 1.50)	(0.50, 10.50)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}24h}$: area under the response versus time curve from time zero to 24 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in serum; T_{Emax}: time to reach maximum concentration observed in serum.

Supplemental Table 10. Mean change-from-baseline serum C-peptide in multiple- dose phase study.

Parameters	Placebo	Dose Cohort			
		25 mg	100 mg	300 mg	600 mg
Day 1					
AUC0-24h (mmol.h/L)	39.9± 16.0	45.2 ± 10.2	49.2 ± 11.3	40.4 ± 9.2	47.0 ± 8.9
Emax (mmol/L)	5.02 ± 1.57	4.81 ± 1.44	5.02 ± 0.998	4.93 ± 1.44	5.48 ± 0.90
TEmax (h) 11.00	8.5	8.0	6.0	4.0	1.0
	(0.50, 12.00)	(1.00, 12.00)	(1.00, 11.00)	(1.00, 12.00)	(0.50, 11.00)
Day 8					
AUC0-24h (mmol.h/L)	45.8 ± 19.8	41.2 ± 10.2	55.1 ± 9.0	40.6 ± 10.4	52.9 ± 11.0
Emax (mmol/L)	5.52 ± 2.03	4.02 ± 1.34	5.54 ± 1.40	4.46 ± 0.64	5.51 ± 1.60
TEmax (h)	11.25	10.75	11.5	11.0	1.5
	(0.50, 12.00)	(1.00, 12.00)	(1.00, 12.00)	(6.00, 12.00)	(0.50, 12.00)

Data are expressed as mean ± S.D.

T_{Emax} is presented as median (minimum, maximum).

$AUC_{0\text{-}24h}$: area under the response versus time curve from time zero to 24 h were calculated using the change-from-baseline data; E_{max}: maximum concentration observed in serum; T_{Emax}: time to reach maximum concentration observed in serum.