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Pharmacokinetic, pharmacodynamic, and efficacy profiles of alogliptin, a novel inhibitor of dipeptidyl peptidase-4, in rats, dogs, and monkeys

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ABSTRACT

The aim of the present research was to characterize the pharmacokinetic, pharmacodynamic, and efficacy profiles of alogliptin, a novel quinazolinone-based dipeptidyl peptidase-4 (DPP-4) inhibitor. Alogliptin potently inhibited human DPP-4 *in vitro* (mean IC_{50}, ~ 6.9 nM) and exhibited > 10,000-fold selectivity for DPP-4 over the closely related serine proteases DPP-2, DPP-8, DPP-9, fibroblast activation protein/seprase, prolyl endopeptidase, and tryptase (IC_{50} > 100,000 nM). Absolute oral bioavailability of alogliptin in rats, dogs, and monkeys was 45%, 86%, and 72% to 88%, respectively. After a single oral dose of alogliptin, plasma DPP-4 inhibition was observed within 15 min and maximum inhibition was > 90% in rats, dogs, and monkeys; inhibition was sustained for 12 h in rats (43%) and dogs (65%) and 24 h in monkeys (> 80%). From E_{max} modeling, 50% inhibition of DPP-4 activity was observed at a mean alogliptin plasma concentration (EC_{50}) of 3.4 to 5.6 ng/ml (10.0 to 16.5 nM) in rats, dogs, and monkeys. In Zucker *fa/fa* rats, a single dose of alogliptin (0.3, 1, 3, and 10 mg/kg) inhibited plasma DPP-4 (91% to 100% at 2 h and 20% to 66% at 24 h), increased plasma GLP-1 (2- to 3-fold increase in $AUC_{0–20\ min}$) and increased early-phase insulin secretion (1.5- to 2.6-fold increase in $AUC_{0–20\ min}$) and reduced blood glucose excursion (31%–67% decrease in $AUC_{0–90\ min}$) after oral glucose challenge. Alogliptin (30 and 100 mg/kg) had no effect on fasting plasma glucose in normoglycemic rats. In summary, these data suggest that alogliptin is a potent and highly selective DPP-4 inhibitor with demonstrated efficacy in Zucker *fa/fa* rats and potential for once-daily dosing in humans.

1. Introduction

The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released by gut endocrine cells in response to a meal and play an important role in glucose homeostasis (Drucker, 2007). Both peptides regulate blood glucose levels by stimulating glucose-dependent insulin secretion. GLP-1 also inhibits glucose-dependent glucagon secretion, delays gastric emptying, enhances satiety, and regulates food intake (Drucker, 2007). In addition, both peptides have been shown to exert direct trophic effects on beta-cell proliferation, differentiation, growth, and survival in preclinical studies (Meier and Nauck, 2006; Drucker, 2007). GLP-1 and GIP are rapidly degraded and inactivated *in vivo*, primarily by the enzyme dipeptidyl peptidase-4 (DPP-4), a serine aminopeptidase present in soluble form in the circulation and also expressed on a variety of cell types (Lambeir et al., 2003). Cleavage of GLP-1 and GIP by DPP-4 yields N-terminally truncated forms of these peptides that are inactive or even antagonistic and is largely responsible for the short plasma half-lives of these hormones (Mentlein et al., 1993; Mentlein, 1999; Thorens et al., 1993; Gault et al., 2002).

In an effort to harness the therapeutic potential of the incretin hormones, DPP-4-resistant GLP-1 analogs (incretin mimetics) and DPP-4 inhibitors (incretin enhancers) have emerged as new classes of antihyperglycemic agents for the treatment of patients with type 2 diabetes (Mest and Mentlein, 2005; Drucker and Nauck, 2006). Studies conducted with several orally administered DPP-4 inhibitors have revealed that inhibition of DPP-4 increases active (intact) GLP-1 and GIP plasma concentrations and improves glycemic control in both animal models of type 2 diabetes (Pederson et al., 1998; Balkan et al., 1999; Ahren et al., 2000; Sudre et al., 2002; Burkey et al., 2005; Pospisilik et al., 2002; Mu et al., 2006; Yasuda et al., 2006) and patients with type 2 diabetes (Ahren et al., 2002; Herman et al., 2007; Kleppinger and Helms, 2007).

Alogliptin benzoate (formerly known as SYR-322) is a novel, orally available, quinazolinone-based, noncovalent DPP-4 inhibitor under development to improve glycemic control in patients with type 2 diabetes (Feng et al., 2007). Preliminary *in vitro* studies have revealed that alogliptin exhibits > 10,000-fold selectivity for DPP-4 over the closely-related serine proteases DPP-8 and DPP-9 (Feng et al., 2007). High selectivity for DPP-4 over DPP-8/DPP-9 may be an important characteristic for this class of agents, as a DPP-8/DPP-9-selective

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inhibitor has been reported to be associated with multiorgan toxicity in rats and dogs and attenuation of human T-cell activation *in vitro* (Lankas et al., 2005). Alogliptin has been shown to exhibit favorable pharmacokinetic, pharmacodynamic, and safety pharmacology profiles in preliminary preclinical studies (Feng et al., 2007). In addition, a single dose of alogliptin has been shown to significantly improve early-phase insulin secretion and oral glucose tolerance in Wistar fatty rats (Feng et al., 2007).

In the current research, the *in vitro* selectivity of alogliptin and the pharmacokinetic and pharmacodynamic profiles of a single oral dose of alogliptin in rats, dogs, and monkeys were comprehensively characterized. In addition, the dose-dependent effects of alogliptin on plasma DPP-4 activity, plasma GLP-1 levels, insulin secretion, and oral glucose tolerance were determined in Zucker *fa/fa* rats, a rodent model of insulin resistance that exhibits hyperinsulinemia and glucose intolerance. Finally, the potential for alogliptin to cause hypoglycemia was investigated by assessing the effects of a single oral dose on fasting plasma glucose and insulin levels in normoglycemic rats.

2. Materials and methods

2.1. Chemicals

Alogliptin (2-[[6-[(3*R*)-3-amino-1-piperidinyl]-3,4-dihydro-3-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]methyl]benzonitrile) was synthesized as the benzoate (Fig. 1), hydrochloride, trifluoroacetate, and tosylate salt forms, as previously described (Feng et al., 2007). Vildagliptin (1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine) and sitagliptin ((2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine) were synthesized using published synthetic routes (Villhauer et al., 2003; Kim et al., 2005). Nateglinide was purchased from Yamanouchi Pharmaceutical Co, Ltd (Tokyo, Japan).

2.2. Enzyme inhibition assays

Recombinant human DPP-4, DPP-2, DPP-8, DPP-9, fibroblast activation protein (FAP)/seprase, and prolyl endopeptidase (PREP) were expressed using baculovirus and purified. Recombinant human tryptase was purchased from Promega Corp (Madison, WI). Inhibition of DPP-4 activity was measured as previously described (Feng et al., 2007). Briefly, the recombinant enzyme or plasma sample was pre-incubated with serial concentrations of inhibitor. The reaction was started by adding Ala-Pro-7-amido-4-trifluoromethylcoumarin (Bachem, King of Prussia, PA), and liberation of the fluorescent 7-amino-4-trifluoromethylcoumarin product was monitored at Ex400 nm and Em505 nm with a SpectraMax Gemini spectrofluorometer (Molecular Devices, Sunnyvale, CA). Similar methods were used for the DPP-2, DPP-8, DPP-9, and FAP/seprase assays. For the PREP assay, the reaction was started with benzyloxycarbonyl Gly-Pro-7-amido-4-methylcoumarin (Bachem), and liberation of the fluorescent 7-amino-4-methylcoumarin product was monitored at Ex375 nm and Em460 nm. For the tryptase assay, the reaction was started with benzyloxycarbonyl-lysine thiobenzyl ester (Bachem), and liberation of the thiobenzyl alcohol product was detected using the chromogenic indicator 5,5′-dithio-bis(2-nitrobenzoic acid) and monitored at 405 nm.

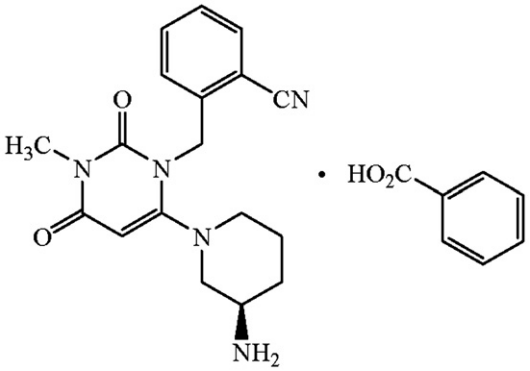

Fig. 1. Chemical structure of alogliptin benzoate (2-[[6-[(3*R*)-3-amino-1-piperidinyl]-3,4-dihydro-3-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]methyl]benzonitrile monobenzoate).

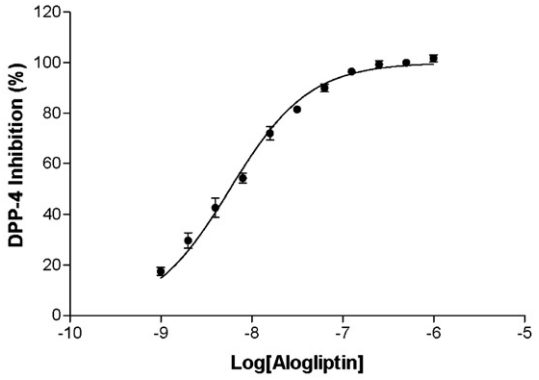

Fig. 2. Concentration-dependent inhibition of the enzymatic activity of recombinant human DPP-4 by alogliptin. Recombinant enzyme was pre-incubated with serial concentrations of alogliptin. The reaction was then started by adding Ala-Pro-7-amido-4-trifluoromethylcoumarin, and liberation of the fluorescent 7-amino-4-trifluoromethylcoumarin product was monitored at Ex400 nm and Em505 nm. Data are mean and S.E.M. (n=4).

2.3. Animals

Male Sprague–Dawley rats and non-naïve male beagle dogs were obtained from Charles River Laboratories (Worchester, MA) and non-naïve male cynomolgus monkeys from MPI Research, Inc, (Mattawan, MI) for the pharmacokinetic/pharmacodynamic studies. Male Sprague–Dawley rats were purchased from Clea Japan Inc (Tokyo, Japan) for the study of the hypoglycemic potential of alogliptin. Male HsdHlr:ZUCKER Lepr^{fa} (Zucker *fa/fa*) rats were purchased from Harlan, Inc (Indianapolis, IN). The care and use of the animals and the experimental protocols used in this research were approved by the International Animal Care and Use Committee at Sidney Kimmel Cancer Center (San Diego, CA) or the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company, Ltd. (Osaka, Japan).

2.4. Pharmacokinetic and pharmacodynamic profiling in rats, dogs, and monkeys

Male Sprague–Dawley rats (250–290 g) were fasted overnight prior to administration of alogliptin intravenously (i.v.) via a catheter in the jugular vein (1 mg/kg; n = 3) or by oral gavage (p.o.; 10 mg/kg; n = 3). Male beagle dogs (8–12 kg) were fasted overnight prior to administration of alogliptin i.v. via a catheter in a saphenous vein (1 mg/kg; n = 3) or p.o.

Table 1
Inhibition of recombinant human DPP-4 and related serine proteases

Enzyme	IC_{50} (nM)[a]		
	Alogliptin	Vildagliptin	Sitagliptin
DPP-4	6.9±1.5	23.8±1.6	12.1±0.8
DPP-2	>100,000	>100,000	>50,000
DPP-8	>100,000	1,400±200	19,000±2000
DPP-9	>100,000	81.5±8.1	62,000±4000
PREP	>100,000	>50,000	>100,000
FAP/seprase	>100,000	73,000±8000	>100,000
Tryptase	>390,000	>200,000	>400,000

DPP = dipeptidyl peptidase; PREP = prolyl endopeptidase; FAP = fibroblast activation protein.
[a] Each assay was performed at least 4 times. Values are expressed as mean and S.E.M.

(3 mg/kg; n = 5). Male cynomolgus monkeys (3.6–5.6 kg) were fasted overnight prior to administration of alogliptin i.v. via a venous catheter (0.1 mg/kg; n = 4) or p.o. (2, 10, and 30 mg/kg; n = 4 per dose level). Doses administered to rats and dogs are shown as the absolute dose form and doses administered to monkeys are shown as the free base form. For all species, blood samples were collected prior to dosing and over 24 h

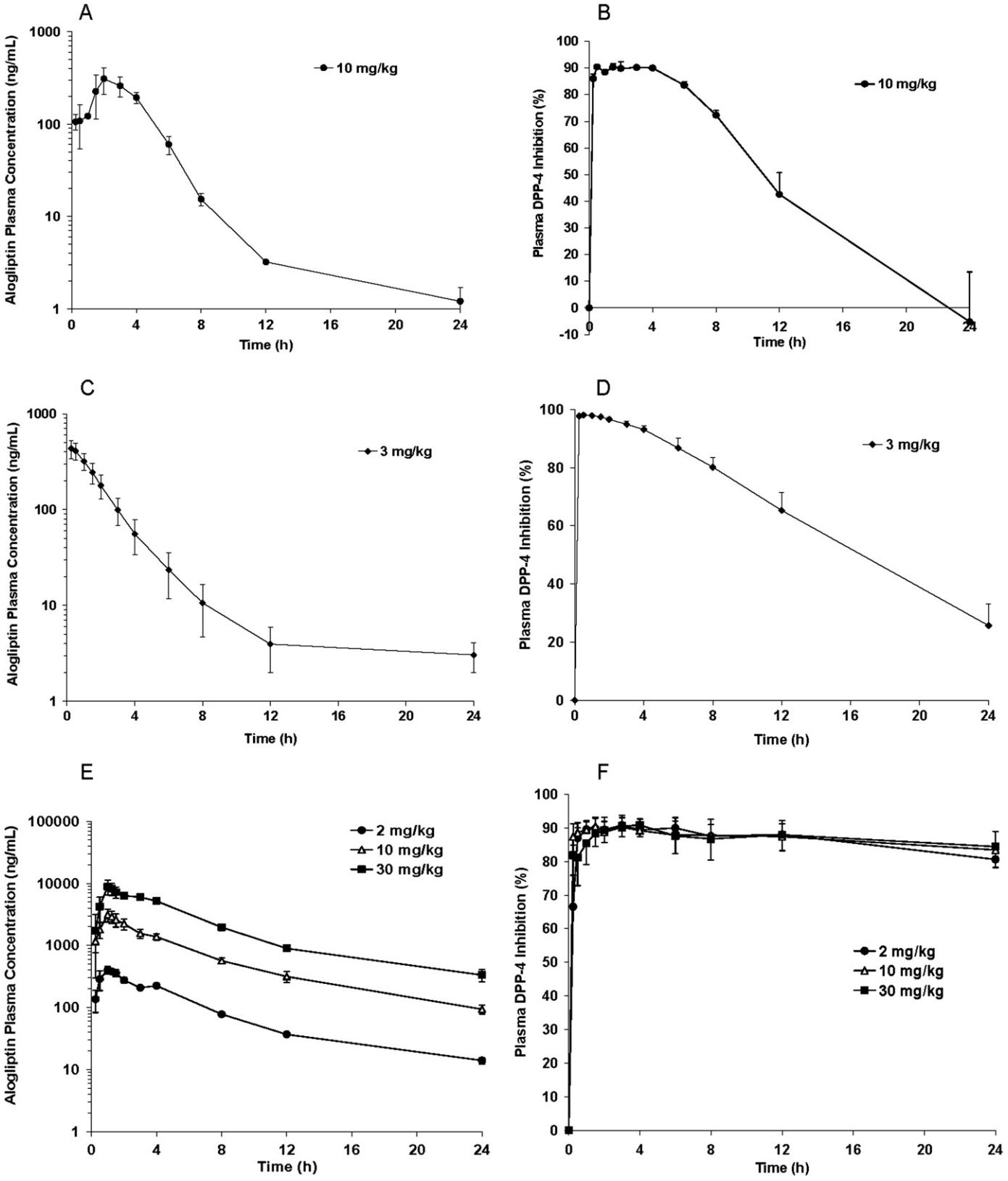

Fig. 3. Plasma concentration profile of alogliptin and plasma DPP-4 inhibition by alogliptin in rats (A, B), dogs (C, D), and monkeys (E, F). Animals were fasted overnight and then administered a single dose of alogliptin by oral gavage. Blood samples were collected prior to dosing and over 24 h postdose for the measurement of alogliptin plasma concentrations and plasma DPP-4 inhibition. Doses of alogliptin administered are indicated in the data labels. Data are mean and S.E.M. (n=3 for rats; n=5 for dogs; n=4 for monkeys).

Table 2
Summary of mean (S.E.M.) pharmacokinetic parameters for alogliptin after administration of a single oral or intravenous dose in rats, dogs, and monkeys

Species	Route	*n*	Dose[a] (mg/kg)	C_{max}[b] (ng/ml)	T_{max} (h)	AUC_{Extrap} (ng·h/ml)	$t_{1/2}$ (h)	CL (ml/kg/h)	Vd_{ss} (ml/kg)	*F* (%)
Rat	p.o.	3	10	393±27	2.3±0.3	1240±67	2.8±1.4	–	–	45±3
	i.v.	3	1	374±8	–	263±17	1.4±0.3	2972±176	3516±376	–
Dog	p.o.	5	3	447±92	0.4±0.1	950±261	1.5±0.2	–	–	86±24
	i.v.	3	1	447±35	–	372±39	1.5±0.0	2435±222	3853±200	–
Monkey	p.o.	4	2	467±35	1.0±0.2	2331±99	7.0±0.4	–	–	72±3
	p.o.	4	10	3208±715	1.0±0.2	16,633±1260	5.7±0.1	–	–	87±7
	p.o.	4	30	9600±2079	2.1±0.5	53,638±1815	5.5±0.7	–	–	88±3
	i.v.	4	1	2117±302	–	2103±125	5.7±0.9	529±32	2602±280	–

C_{max} = maximum observed plasma concentration; T_{max} = time to reach maximum observed plasma concentration; $t_{1/2}$ = elimination half-life; AUC_{Extrap} = extrapolated area under the plasma concentration–time curve; CL = clearance; Vd_{ss} = volume of distribution at steady state; *F* = oral bioavailability; p.o. = oral; i.v. = intravenous; – = not determined/not applicable.

[a] Dose levels reflect nominal doses of alogliptin used in these studies.

[b] C_{max} for i.v. dose represents an estimate of C_0.

postdose into tubes containing heparin. Alogliptin plasma concentrations were determined by liquid chromatography/tandem mass spectrometry (LC/MS/MS) using an API 4000 triple quadruple mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). The following pharmacokinetic parameters were determined from alogliptin plasma concentrations by noncompartmental methods using Watson© Version 5.4.1 (Thermo Fisher Scientific, Inc., Waltham, MA): maximum observed plasma concentration (C_{max}), time to reach maximum observed plasma concentration (T_{max}), elimination half-life ($t_{1/2}$), extrapolated area under the plasma concentration–time curve (AUC_{Extrap}), clearance (CL), volume of distribution at steady state (Vd_{ss}), and oral bioavailability (*F*). *Ex vivo* plasma DPP-4 activity was also determined. The relationship between alogliptin plasma concentration and *ex vivo* plasma DPP-4 inhibition was explored using an inhibitory effect E_{max} model using WinNonLin® Professional Version 4.1 (Pharsight Corp., Mountain View, CA).

2.5. Pharmacodynamic effects in Zucker fa/fa rats

Eight-week-old male Zucker *fa/fa* rats were fed a standard commercial diet and given water *ad libitum*. After an overnight fast (at least 12 h), rats were divided into 5 treatment groups based on body weight and fasting plasma glucose levels ($n = 8$ per group) and administered vehicle alone (0.5% carboxymethylcellulose) or alogliptin (doses shown as free base form) at 0.3, 1, 3, or 10 mg/kg by single bolus oral gavage (5 ml/kg dose volume). At 30 min postdose, rats were given a glucose solution (1 g/kg, 2 ml/kg dose volume). At -30 (pre-drug), 0 (pre-glucose), 10, 20, 40, 60, and 90 min, blood samples were collected from the tail vein into collection tubes containing lithium heparin. Blood glucose concentrations were analyzed up to 90 min after glucose load using the Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN). Plasma GLP-1 concentrations were analyzed up to 20 min after glucose load using a GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, Salem, NH) and plasma insulin concentrations were analyzed up to 60 min after glucose load using an insulin ELISA kit (Crystal Chem, Downers Grove, IL), according to the manufacturers' instructions.

Table 3
Relationship between alogliptin plasma concentration and plasma DPP-4 inhibition in rats, dogs, and monkeys

Species	Dose[a] (mg/kg)	*N*	E_0 (%)	EC_{50} (ng/ml)	E_{max} (%)
			Mean (%CV)	Mean (%CV)	Mean (%CV)
Rat	10	3	9.1 (11.3)	3.4 (50.2)	107.7 (7.0)
Dog	3	3	2.8 (9.8)	4.9 (48.6)	97.9 (11.3)
Monkey[b]	2, 10, 30	4	10.9 (64.7)	5.6 (107.1)	99.5 (1.7)

E_0 = baseline effect; EC_{50} = alogliptin plasma concentration at which DPP-4 activity is inhibited by 50%; E_{max} = maximum percent inhibition; %CV = percent coefficient of variation.

[a] Dose levels reflect nominal doses of alogliptin used in these studies.

[b] Data for all 3 doses were used in model.

In satellite groups of fasted Zucker *fa/fa* rats, alogliptin was administered by single bolus oral gavage at 0.3, 1, 3, 10 mg/kg ($n = 4$ per group; 5 ml/kg dose volume). Blood samples were collected into collection tubes containing lithium heparin at 0 (predose) and at 10, 20, and 40 min and 1, 2, 4, 8, 14, and 24 h postdose. At each time point, alogliptin concentrations in plasma were determined by LC/MS/MS (limit of quantitation, 0.5 ng/ml), and pharmacokinetic analysis was performed by noncompartmental analysis using Watson© Version 7.1.0.01 (Thermo Fisher Scientific, Inc., Waltham, MA). Plasma DPP-4 activity was also determined at each time point.

2.6. Effects on fasting plasma glucose levels in normoglycemic rats

Male Sprague–Dawley rats were fed a commercial diet (CE-2) and given water *ad libitum*. At 7 weeks of age, rats were divided into 5 groups ($n = 6$ per group) based on body weight and fasted overnight. Each group was administered vehicle alone (0.5% methylcellulose), alogliptin (doses shown as free base form) at 30 or 100 mg/kg, or nateglinide at 30 or 100 mg/kg p.o. Blood samples were collected from the tail vein prior to dosing (time 0) and at 30, 60, and 120 min postdose. At each time point, plasma glucose levels were determined enzymatically using the Autoanalyzer 7080 (Hitachi, Tokyo, Japan), and plasma insulin levels were determined by radioimmunoassay (LINCO Research, St Charles, MO).

2.7. Statistical analysis

Data are expressed as mean and S.E.M. In the Zucker *fa/fa* rat study, baseline (0 min)-adjusted area under the concentration–time curve from time 0 to 90 min after glucose load ($AUC_{0–90\ min}$) for blood glucose, $AUC_{0–20\ min}$ for plasma GLP-1, and baseline (0 min)-adjusted $AUC_{0–20\ min}$ for plasma insulin were calculated using the linear trapezoidal rule. In the normoglycemic rat study, baseline (0 min)-adjusted $AUC_{0–120\ min}$ for plasma glucose was also calculated using the linear trapezoidal rule. In the Zucker *fa/fa* rat study, the differences between treatment groups were analyzed by the Student's *t* test or one-way analysis of variance, with *P* values < 0.05 (two-tailed) considered statistically significant. In the normoglycemic rat study, the statistical significance of the change in baseline-adjusted plasma glucose $AUC_{0–120\ min}$ in alogliptin-treated or nateglinide-treated rats vs. vehicle-treated control rats was determined using a one-tailed Williams' test.

3. Results

3.1. In vitro potency and selectivity of alogliptin

The ability of alogliptin to inhibit the enzymatic activity of DPP-4 and closely related serine proteases was investigated *in vitro*. As shown in Fig. 2 and Table 1, alogliptin potently inhibited human recombinant DPP-4, with a mean (S.E.M.) half maximal inhibitory

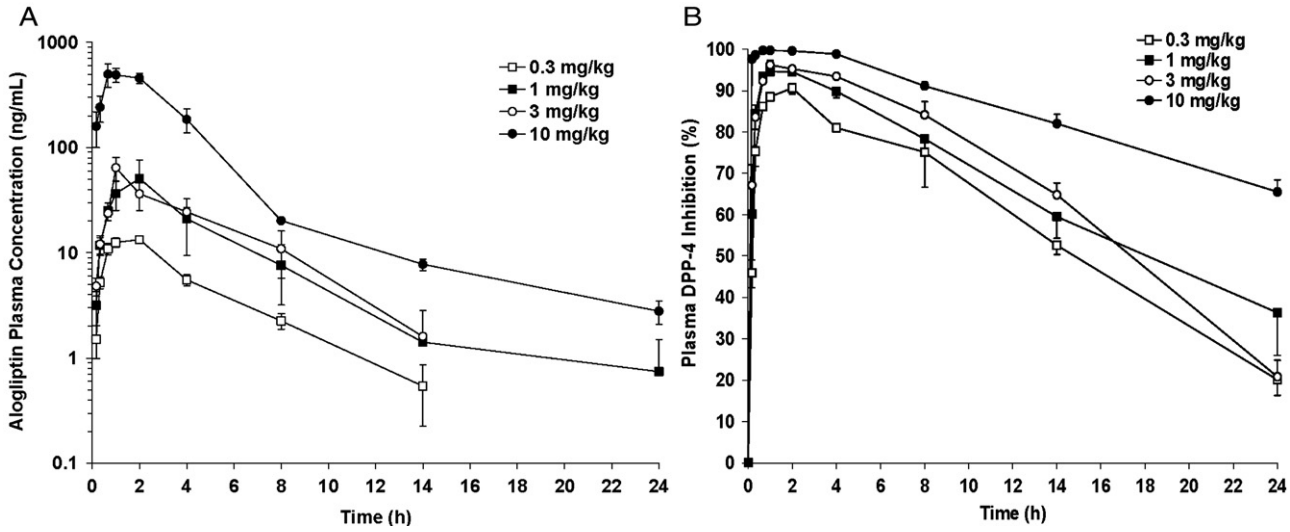

Fig. 4. Plasma concentration profile of alogliptin (A) and plasma DPP-4 inhibition by alogliptin (B) in Zucker *fa/fa* rats. Animals were fasted overnight and then administered a single dose of alogliptin by oral gavage. Blood samples were collected prior to dosing (0 min) and at 10, 20, 40 min and 1, 2, 4, 8, 14, and 24 h postdose for the measurement of alogliptin plasma concentrations and plasma DPP-4 inhibition. Doses of alogliptin administered are indicated in the data labels. Data are mean and S.E.M. (n=4).

concentration (IC_{50}) of 6.9 ± 1.5 nM. Consistent with these results, alogliptin also potently inhibited DPP-4 activity in rat and dog plasma, with an IC_{50} in the range of 6 to 10 nM. In contrast, alogliptin exhibited no inhibitory activity against recombinant human DPP-2, DPP-8, DPP-9, FAP/seprase, PREP, or tryptase over the concentration ranges tested, indicating that alogliptin is > 10,000-fold more selective for DPP-4 over these closely related serine proteases. Furthermore, alogliptin was more selective for DPP-4 compared with the inhibitors vildagliptin or sitagliptin in these assays (Table 1).

3.2. Pharmacokinetic profile of alogliptin in rats, dogs, and monkeys

The pharmacokinetics of alogliptin were determined after single dose p.o. and i.v. administration to male Sprague–Dawley rats, beagle dogs, and cynomolgus monkeys. Plasma concentrations and pharmacokinetic parameters for alogliptin in these species are summarized in Fig. 3 (p.o.) and Table 2 (p.o. and i.v.), respectively. Maximal plasma concentrations of alogliptin were achieved rapidly across all species after a single oral dose (mean T_{max}, 0.4–2.3 h across species). Alogliptin was eliminated more slowly in monkeys (mean $t_{1/2}$, 5.5–7.0 h) than in rats (mean $t_{1/2}$, 2.8 h) or dogs (mean $t_{1/2}$, 1.5 h). Plasma concentrations of alogliptin were maintained in all species at 24 h after a single oral dose and were dose-dependent in monkeys. The absolute oral bioavailability of alogliptin was higher in dogs (86%) and monkeys (72%–88%) than in rats (45%).

3.3. Pharmacodynamic profile of alogliptin in rats, dogs, and monkeys

Inhibition of plasma DPP-4 activity after administration of a single oral dose of alogliptin was determined in male Sprague–Dawley rats, beagle dogs, and cynomolgus monkeys (Fig. 3). DPP-4 inhibition was first observed at 15 min postdose (first analysis time point), at which time approximately 86%, 98%, and 67% to 87% inhibition was observed in rats, dogs, and monkeys, respectively. In rats, maximum inhibition (90%) was observed as early at 30 min postdose, and inhibition was observed through 12 h postdose (43%) (Fig. 3B). In dogs, maximum inhibition (98%) was first observed at 15 min postdose, and inhibition was observed through 12 h postdose (65%) (Fig. 3D). In monkeys, near maximum inhibition of DPP-4 activity was observed through 24 h postdose (> 80%) for all dose levels (Fig. 3F).

Analysis of the relationship between alogliptin plasma concentration and plasma DPP-4 inhibition using E_{max} modeling revealed that 50% inhibition of DPP-4 activity (EC_{50}) was observed at a mean alogliptin plasma concentration of 3.4, 4.9, and 5.6 ng/ml (10.0, 14.4, and 16.5 nM) in rats, dogs, and monkeys, respectively (Table 3).

3.4. Effect of alogliptin on plasma DPP-4 activity, plasma GLP-1 and insulin levels, and oral glucose tolerance in Zucker fa/fa rats

Alogliptin was rapidly absorbed after administration of a single oral dose to Zucker *fa/fa* rats (Fig. 4A), with a mean T_{max} of 1.0 to 1.4 h observed across the 0.3 to 10 mg/kg dose range. Dose-related increases in mean C_{max} and AUC_{0-t} were observed, the estimates ranging from 14 to 556 ng/ml and 60 to 1998 ng·h/ml, respectively, across doses. Administration of a single oral dose of alogliptin resulted in rapid, almost complete, and sustained inhibition of plasma DPP-4 activity in the Zucker *fa/fa* rats (Fig. 4B). Plasma DPP-4 activity was inhibited in a dose-related manner as early as 20 min postdose (first

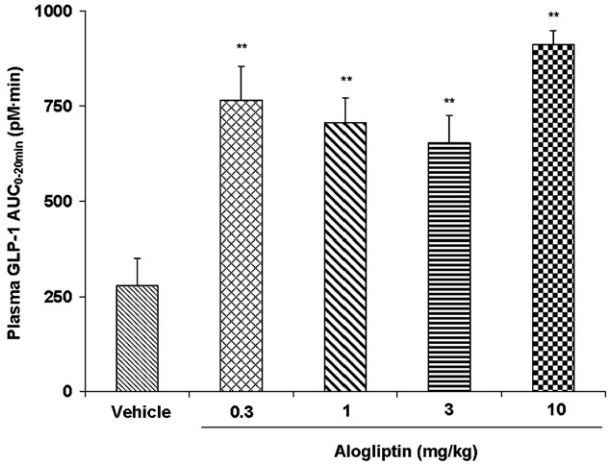

Fig. 5. Effect of a single oral dose of alogliptin on plasma GLP-1 $AUC_{0-20\ min}$ after an oral glucose load in Zucker *fa/fa* rats. Animals were fasted overnight and then administered a single dose of vehicle alone (0.5% carboxymethylcellulose) or alogliptin by oral gavage. At 30 min postdose, the rats were given a glucose solution (1 g/kg). Blood samples were collected prior to dosing (−30 min), prior to glucose load (0 min), and at 10, 20, 40, 60, and 90 min. GLP-1 levels were measured up to 20 min post glucose load. Doses of alogliptin administered are indicated in the data labels. Data are mean and S.E.M. (n=8). **P<0.01 (vs. vehicle control using an unpaired Student's t test).

analysis time point; 10 min before oral glucose load), at which time the mean (S.E.M.) inhibition was 45.9 ± 3.59%, 60.1 ± 11.04%, 67.1 ± 4.94%, and 97.6 ± 0.94% for the 0.3, 1, 3, and 10 mg/kg doses, respectively. Inhibition was maintained throughout the sampling period, with approximately 90.6 ± 1.40%, 94.5 ± 0.74%, 95.2 ± 0.73%, and 99.6 ± 0.04% inhibition at 2 h postdose and 20.1 ± 3.77%, 36.3 ± 10.31%, 20.8 ± 3.98%, and 65.5 ± 2.91% inhibition at 24 h postdose for the 0.3, 1, 3, and 10 mg/kg doses, respectively. Visual inspection of the data across doses revealed that alogliptin plasma concentrations of approximately 5.5 ng/ml (16.2 nM) were sufficient to inhibit plasma DPP-4 activity by at least 80% in Zucker *fa/fa* rats.

No statistically significant difference in plasma GLP-1 levels at predose was observed between each alogliptin dose and vehicle alone; the mean (S.E.M.) value ranged from 49.2 ± 5.3 pM to 64.9 ± 4.3 pM (10 mg/kg) across all groups. Significant increases in mean GLP-1 $AUC_{0-20\ min}$ of approximately 2 fold were observed for the 0.3, 1, and 3 mg/kg doses compared with vehicle alone ($P < 0.01$) (Fig. 5). The maximum effect on GLP-1 exposure was observed for the 10 mg/kg dose, where an approximately 3-fold increase in mean $AUC_{0-20\ min}$ was observed compared with vehicle alone ($P < 0.001$).

Early-phase insulin secretion was increased after a single dose of alogliptin compared with vehicle alone. Plasma insulin levels peaked at 10 min after oral glucose load for all treatment groups (Fig. 6A). For the 0.3, 1, and 3 mg/kg doses, increases of approximately 1.5, 1.5, and 1.8 fold, respectively, in mean baseline-adjusted plasma insulin $AUC_{0-20\ min}$ were observed compared with vehicle alone, but these changes were not statistically significant (Fig. 6B). The maximum effect on early-phase insulin secretion was observed for the 10 mg/kg dose, where a significant increase of approximately 2.6 fold in plasma insulin $AUC_{0-20\ min}$ was observed compared with vehicle alone ($P < 0.05$).

Significant decreases in blood glucose excursion were observed for all alogliptin doses compared with vehicle alone after an oral glucose load (Fig. 6C and D). Mean baseline-adjusted blood glucose $AUC_{0-90\ min}$ was decreased by approximately 31%, 37%, and 41% for the 0.3, 1, and 3 mg/kg doses, respectively, compared with vehicle alone ($P < 0.05$). As observed for plasma insulin levels, the maximum effect on glucose excursion was observed for the 10 mg/kg dose, whereas an approximately 67% decrease in blood glucose $AUC_{0-90\ min}$ was observed when compared with vehicle alone ($P < 0.01$).

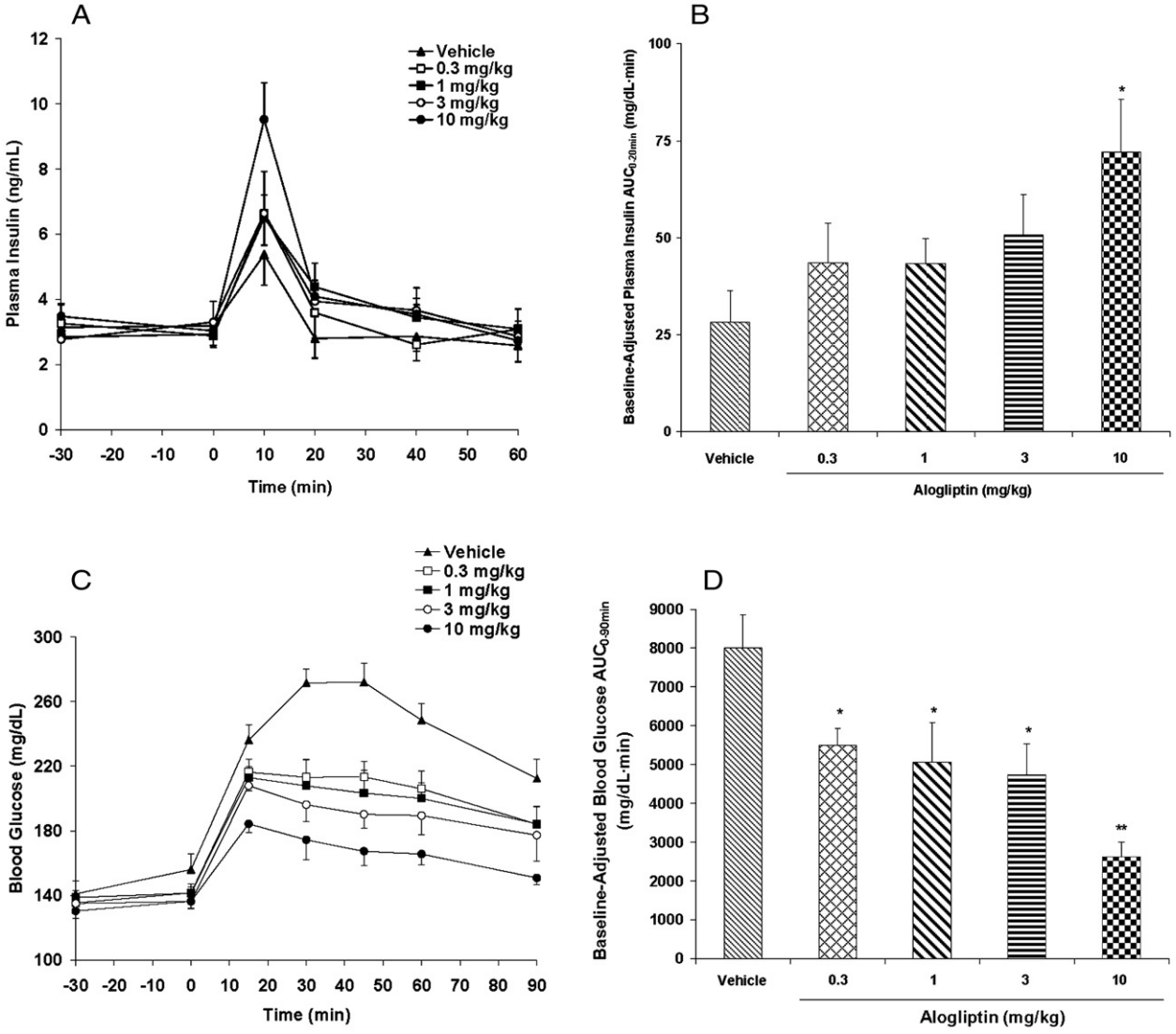

Fig. 6. Effects of a single oral dose of alogliptin on plasma insulin levels (A), baseline-adjusted plasma insulin $AUC_{0-20\ min}$ (B), blood glucose levels (C), and baseline-adjusted blood glucose $AUC_{0-90\ min}$ (D) after an oral glucose load in Zucker *fa/fa* rats. Animals were fasted overnight and then administered a single dose of vehicle alone (0.5% carboxymethylcellulose) or alogliptin by oral gavage. At 30 min postdose, the rats were given a glucose solution (1 g/kg). Blood samples were collected prior to dosing (−30 min), prior to glucose load (0 min), and at 10, 20, 40, 60, and 90 min. Plasma insulin levels were measured up to 60 min and blood glucose levels up to 90 min post glucose load. Doses of alogliptin administered are indicated in the data labels. Data are mean and S.E.M. (n=8). *P<0.05 and **P<0.01 (vs. vehicle control using an unpaired Student's t test).

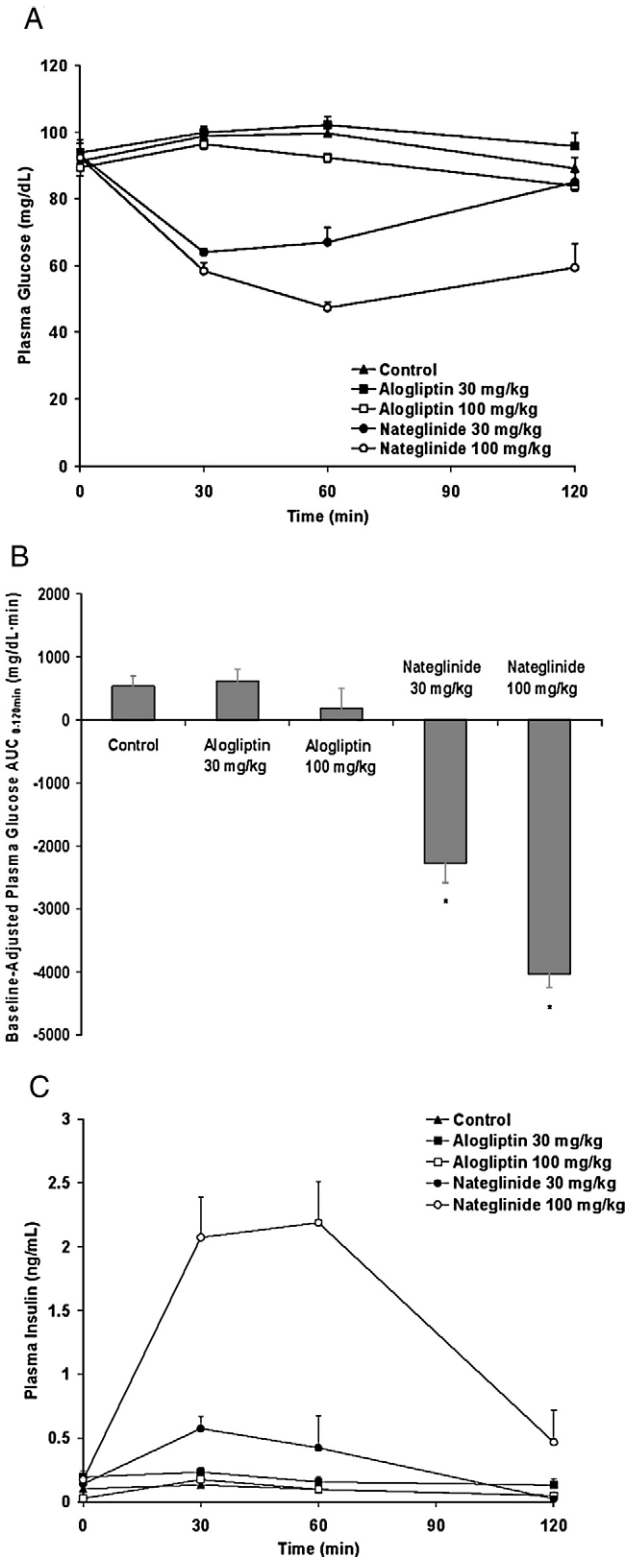

Fig. 7. Effects of alogliptin on plasma glucose levels (A), baseline-adjusted plasma glucose $AUC_{0-120\ min}$ (B), and plasma insulin levels (C) in Sprague–Dawley rats. After an overnight fast, rats were administered vehicle alone (0.5% methylcellulose), alogliptin, or nateglinide by oral gavage. Blood samples were collected from the tail vein prior to dosing (0 min) and at 30, 60, and 120 min postdose for the measurement of fasting plasma glucose and insulin levels. Doses of alogliptin and nateglinide administered are indicated in the data labels. Data are mean and S.E.M. (n=6). *$P \leq 0.025$ (vs. vehicle control using a one-tailed Williams' test).

3.5. Effect of alogliptin on fasting plasma glucose levels in normoglycemic rats

The effect of a single oral dose of alogliptin and nateglinide on fasting plasma glucose levels in normoglycemic male Sprague–Dawley rats was determined. Nateglinide (30 or 100 mg/kg) dose-dependently lowered plasma glucose below normal fasting levels; a statistically significant decrease in baseline-adjusted plasma glucose $AUC_{0-120\ min}$ was observed in the rats treated with nateglinide 30 or 100 mg/kg ($P \leq 0.025$ vs. vehicle control) (Fig. 7A and B). Nateglinide also dose-dependently increased plasma insulin levels in these rats (Fig. 7C). In contrast, a single dose of alogliptin (30 or 100 mg/kg) had no effect on fasting plasma glucose or insulin levels (Fig. 7A–C).

4. Discussion

Inhibitors of the enzyme DPP-4 represent a new class of antihyperglycemic agents that act by increasing active levels of the incretin hormones GLP-1 and GIP (Mest and Mentlein, 2005; Drucker and Nauck, 2006). Alogliptin is a novel, orally available, quinazolinone-based, noncovalent DPP-4 inhibitor under development to improve glycemic control in patients with type 2 diabetes. The data presented in this report demonstrate that alogliptin is a potent and highly selective inhibitor of DPP-4 *in vitro*, exhibiting a mean IC_{50} of approximately 6.9 nM and > 10,000-fold selectivity for DPP-4 over the closely related serine proteases DPP-2 (also known as quiescent cell proline peptidase [QPP]), DPP-8, DPP-9, FAP/seprase, PREP, and tryptase. Furthermore, alogliptin appears to be more selective for DPP-4 compared with the inhibitors vildagliptin or sitagliptin. The mean IC_{50} values determined for vildagliptin and sitagliptin were similar to those previously reported in the literature for these compounds (Villhauer et al., 2003; Brandt et al., 2005; Burkey et al., 2006; Kim et al., 2005). Although the *in vivo* functions of DPP-8 and DPP-9 have yet to be determined, a DPP-8/DPP-9-selective inhibitor was reported to be associated with multiorgan toxicity in rats and dogs and attenuation of human T-cell activation *in vitro*, whereas a DPP-4-selective inhibitor did not cause these effects (Lankas et al., 2005). These findings suggest that selectivity for DPP-4 may be important to ensure an optimal safety profile for this new class of antihyperglycemic agents.

A single oral dose of alogliptin exhibited favorable pharmacokinetic and pharmacodynamic profiles in rats, dogs, and, monkeys. Alogliptin was absorbed rapidly (mean T_{max}, 0.4 to 2.3 h across species) and exhibited an absolute oral bioavailability of 45% to 88% across species. Consistent with the rapid absorption of alogliptin, inhibition of plasma DPP-4 activity occurred rapidly (15 min postdose) after administration of a single oral dose in all species. Almost complete inhibition of DPP-4 activity in plasma was observed, with maximum inhibition being ≥ 90% in all species. Furthermore, plasma DPP-4 inhibition was sustained for 12 h in rats (43%) and dogs (65%) and for 24 h in monkeys (> 80%), supportive of a once-daily dosing regimen of alogliptin. From the E_{max} modeling, the EC_{50} determined for alogliptin ranged from 3.4 to 5.6 ng/ml (10.0 to 16.5 nM) across species, indicating that alogliptin is a potent inhibitor of DPP-4 *in vivo* and similar to the IC_{50} of approximately 6.9 nM determined *in vitro*. The EC_{50} for alogliptin was in the same order of magnitude in the rat, dog, and monkey, suggesting that there is no significant interspecies difference in the sensitivity to this compound.

Impaired insulin secretion in patients with type 2 diabetes is often characterized by a decreased first-phase insulin response, which leads to glucose intolerance and postprandial hyperglycemia (Taylor et al., 1994). Zucker *fa/fa* rats, which have defects in both insulin secretion and insulin sensitivity that lead to hyperinsulinemia and glucose intolerance (Ionescu et al., 1985; Pénicaud et al., 1991), represent a well-characterized model of insulin resistance for assessing the *in vivo* efficacy of DPP-4 inhibitors. Studies with DPP-4 inhibitors such as isoleucine-thiazolidide, NVP-DPP728, FE999011, vildagliptin, and

E3024 revealed that these inhibitors cause significant reduction in glucose excursion concomitant with elevations in plasma insulin and active GLP-1 levels in an oral glucose tolerance test in normal and obese Zucker *fa/fa* rats (Pederson et al., 1998; Balkan et al., 1999; Sudre et al., 2002; Villhauer et al., 2003; Burkey et al., 2005; Yasuda et al., 2006). Consistent with these studies, alogliptin also improved oral glucose tolerance in obese Zucker *fa/fa* rats after a single oral dose. After administration of alogliptin to Zucker *fa/fa* rats over the 0.3 to 10 mg/kg dose range, inhibition of plasma DPP-4 activity was dose-dependent, rapid (within 15 min postdose), almost complete (>90% inhibition across all doses), and sustained (>65% inhibition at 24 h after 10 mg/kg dose). Alogliptin plasma concentrations of approximately 5.5 ng/ml (16.2 nM) were sufficient to inhibit plasma DPP-4 activity by at least 80%, demonstrating the potency of alogliptin as a DPP-4 inhibitor in this rodent model. Inhibition of plasma DPP-4 activity was associated with increased GLP-1 levels and early-phase insulin secretion and reduced blood glucose excursion in response to oral glucose challenge across the entire 0.3 to 10 mg/kg dose range. The effects on GLP-1 levels, early-phase insulin secretion, and blood glucose excursion were dose dependent, and maximal effects were observed with the 10 mg/kg dose (3-fold increase in mean GLP-1 $AUC_{0-20\ min}$, 2.6-fold increase in mean insulin $AUC_{0-20\ min}$, and 67% decrease in mean glucose $AUC_{0-90\ min}$ vs. vehicle control).

It is likely that the insulin-stimulating and glucose-lowering effects of alogliptin in Zucker *fa/fa* rats were mediated, at least in part, by increasing levels of GLP-1 which is known to increase glucose-dependent insulin secretion of pancreatic beta-cells. Indeed, alogliptin increased GLP-1 levels after glucose load in the Zucker *fa/fa* rats. In addition, the possible role of other peptide substrates of DPP-4, beyond GLP-1, that influence insulin secretion and glucose turnover in mediating the glucose-lowering effects of alogliptin cannot be ruled out. The DPP-4 inhibitor valine pyrrolidide was at least partially effective in knock-out mice that lacked either the GLP-1 or GIP receptor, suggesting that both GLP-1 and GIP mediate the glucose-lowering effects of DPP-4 inhibitors, at least in rodents (Marguet et al., 2000; Hansotia et al., 2004). Furthermore, DPP-4 inhibition in C57BL/6J mice augmented insulin secretion in response to not only exogenously administered GLP-1 and GIP but also the neuropeptides pituitary adenylate cyclase-activating polypeptide 38 and gastrin-releasing peptide (Ahren and Hughes, 2005). It is also possible that insulin-independent actions of circulating GLP-1, such as the suppression of pancreatic glucagon secretion and retardation of gastric emptying, contributed to the glucose-lowering effects of alogliptin in the Zucker *fa/fa* rats. Alogliptin has been shown to decrease plasma glucagon levels in *ob/ob* mice, another obese rodent model of type 2 diabetes (Moritoh et al., 2007). Although administration of exogenous GLP-1 has been shown to delay gastric emptying (Drucker, 2007), any such effect of DPP-4 inhibitors has yet to be demonstrated. In a recent report, administration of the DPP-4 inhibitor vildagliptin to patients with type 2 diabetes did not alter the rate of nutrient absorption or delivery to the systemic circulation (Vella et al., 2007).

In contrast to nateglinide, a single oral dose of alogliptin had no effect on fasting plasma glucose and insulin levels in normoglycemic rats at doses that exceeded those that improved oral glucose tolerance in Zucker *fa/fa* rats. These results are consistent with the glucose-dependent mechanism of action of alogliptin, which contrasts with the glucose-independent stimulatory effect of nateglinide on insulin secretion. Because of its glucose-dependent effects, it is anticipated that alogliptin will be associated with a lower risk of hypoglycemia in patients with type 2 diabetes than other classes of antidiabetic agents, as has been reported for other DPP-4 inhibitors (Herman et al., 2007; Kleppinger and Helms, 2007).

In summary, alogliptin is a potent and highly selective inhibitor of DPP-4. The pharmacokinetic and pharmacodynamic profiles of alogliptin in preclinical species are supportive of a once-daily dosing regimen. A single oral dose of alogliptin increased plasma GLP-1 levels, augmented early-phase insulin secretion, and improved oral glucose tolerance in Zucker *fa/fa* rats, a rodent model of insulin resistance, but had no effect on fasting glucose levels in normoglycemic rats. Taken together, the results of these studies indicate that alogliptin may represent a promising new treatment option for type 2 diabetes. Clinical studies are currently underway to investigate the efficacy and safety of alogliptin in patients with this disease.

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