Poster Board LBSU-1075

ZYDPLA1, a Novel Long-Acting DPP-4 Inhibitor

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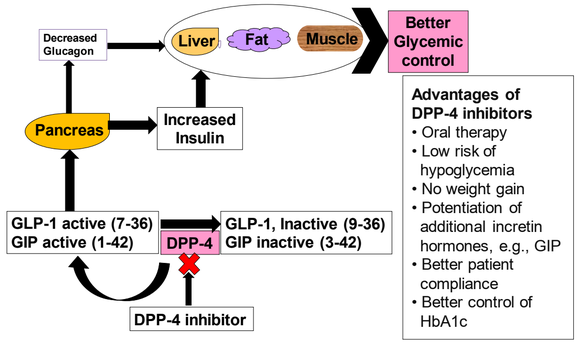
ABSTRACT

DPP-4 inhibitors inhibit degradation of glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), the endogenous incretin hormones responsible for stimulating glucose-dependent insulin secretion. ZYDPLA1 is a novel and potent DPP-4 inhibitor under clinical development for the treatment of type 2 diabetes and has shown potential for once-a-week administration in humans. The *in vitro* effect of ZYDPLA1 was assessed using recombinant DPP-4 enzyme. ZYDPLA1 competitively inhibited DPP-4 activity *in vitro* with an IC_{50} of 2.99 nM, and Ki of 9.3 nM. The calculated Koff rate for ZYDPLA1 was 5.12×10^{-5} S^{-1}. ZYDPLA1 was more than 8000-fold selective for DPP-4 relative to DPP-8, and DPP-9, and was more than 10000-fold selective relative to fibroblast activation protein *in vitro*. The potency of ZYDPLA1 for DPP-4 inhibition was similar across the species. In C57BL/6J mice, ZYDPLA1 administration showed a potent antihyperglycemic effect in oral glucose tolerance test. This effect was mediated through elevated circulating levels of GLP-1 and insulin. Potent antihyperglycemic effect was also observed in Zucker fatty rats following meal tolerance test. Significant DPP-4 inhibition was observed for more than 48 hours in mice and rats and up to 168 hours in dogs and non-human primates. In conclusion, ZYDPLA1 is a potent and selective inhibitor of DPP-4 that has the potential to become once-a-week therapy for treatment of type 2 diabetes.

BACKGROUND



Advantages of DPP-4 inhibitors
- Oral therapy
- Low risk of hypoglycemia
- No weight gain
- Potentiation of additional incretin hormones, e.g., GIP
- Better patient compliance
- Better control of HbA1c

Inadequate management of hyperglycemia is a major cause of macro- and micro-vascular complications in type 2 diabetes. Hence, there is a need to identify new antidiabetic agents that offer sustained glycemic control. Agonists of the GLP-1 receptor (GLP-1R) have shown good anti-hyperglycemic effects. However, they are all injectable peptides that have short half-life and cause side effects like nausea and vomiting. DPP-4 inhibitors increase circulating levels of GLP-1 and GIP and demonstrate optimum glycemic control without GI side effects. Currently available DPP-4 inhibitors require daily administration for exhibiting *in vivo* efficacy. We aimed to identify a novel long-acting oral DPP-4 inhibitor with potential for once-weekly administration in type 2 diabetic patients.

ZYDPLA1 IS A POTENT, SELECTIVE AND LONG ACTING DPP-4 INHIBITOR

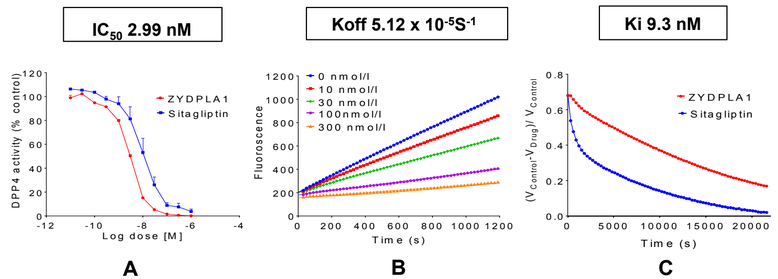


Figure 1. *In vitro* potency of ZYDPLA1: (A) Inhibition of human recombinant DPP-4, (B) Association & (C) Dissociation kinetics for inhibition of rat plasma DPP-4. For the association kinetics experiment, substrate was pre-incubated with ZYDPLA1 (0-300 nM) and enzyme reaction was initiated by adding rat plasma (10 µL). Fluorescence intensities were measured every 20 seconds for total of 1200 seconds. For the dissociation kinetics experiment. rat plasma was pre-incubated with 30 nM ZYDPLA1 and enzyme reaction was initiated by adding the DPP-4 substrate, H-Gly-Pro-AMC. Fluorescence intensities were measured every 5 minute for total of 6 h.

Table 1. Selectivity of ZYDPLA1 over other peptidases

Enzyme	IC_{50} value (µM)
DPP-4	0.0029 ± 0.0001
DPP-8	24.9 ± 2.1
DPP-9	19.7 ± 1.4
FAP	178.0 ± 4.5
Mean ± SEM (n=6)	

Table 2. Effect of ZYDPLA1 on DPP-4 inhibition across species

DPP-4 (Serum)	IC_{50} (nM)
C57 Mice	16.1 ± 3.4
SD Rat	3.2 ± 0.05
Beagle Dog	7.3 ± 0.9
Rhesus Monkey	7.1 ± 0.5
Human	6.8 ± 0.5
Mean ± SEM (n=6)	

Table 3. Percent DPP-4 inhibition by ZYDPLA1 after 24-h dialysis indicating long-lasting action

DPP-4 (Serum)	Sitagliptin (300 nM)	ZYDPLA1 (300 nM)
SD rat	1.6 ± 0.09	57.7 ± 3.2
Human	1.5 ± 0.12	78.7 ± 5.2
Mean ± SEM (n=6)		

ZYDPLA1 INCREASES GLP-1, INSULIN & IMPROVES GLYCEMIC CONTROL

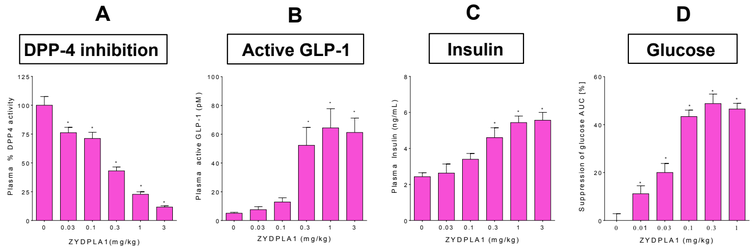


Figure 2. (A) DPP-4 inhibition, (B) Plasma active GLP-1 levels, (C) Plasma insulin levels and (D) Suppression of AUC glucose in OGTT in C57 mice after oral administration of ZYDPLA1. In OGTT, ZYDPLA1 was administered 15 min before the oral glucose load (5 g/kg, p.o.). ZYDPLA1 was administered 60 min before the glucose challenge (5 g/kg, p.o.) and plasma DPP4 % activity, plasma active GLP-1 (ELISA, Epitope Diagnostics Inc, USA) and plasma insulin (ELISA, Crystal Chem Inc, USA) were measured 10 min after glucose load.

ZYDPLA1 IMPROVES GLYCEMIC CONTROL BY INHIBITING DPP-4 IN VIVO

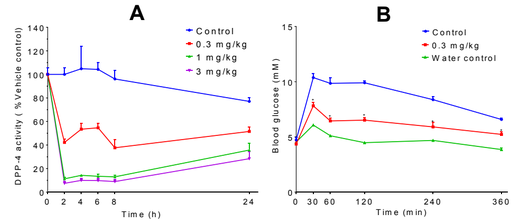


Figure 3. (A) Inhibition of plasma DPP-4 activity after single oral administration of ZYDPLA1 in Wistar rats and (B) Meal tolerance test after oral administration of ZYDPLA1 in Zucker fatty rats. ZYDPLA1 was administered 60 min before the meal challenge (Starch 60%: Sucrose 30%: Lactose 10% w/v in water, 3.5 g/kg/10 ml).

PHARMACOKINETIC-PHARMACODYNAMIC CORRELATION

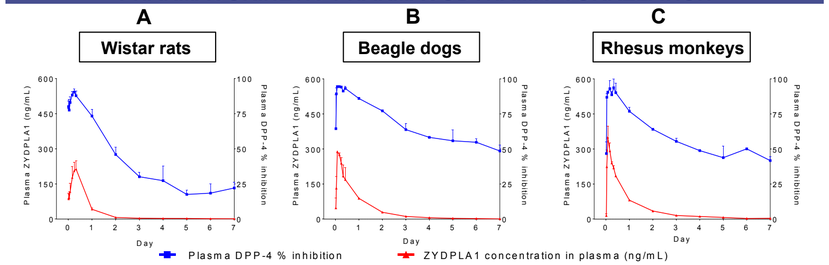


Figure 4. Inhibition of plasma DPP-4 activity and ZYDPLA1 levels in rats (A), dogs (B) and in monkeys (C) after single oral administration of ZYDPLA1. ZYDPLA1 was administered at 2.25 mg/kg in rats, and 2 mg/kg in dogs and monkeys.

ZYDPLA1 PHARMACOKINETICS IN PRECLINICAL SPECIES AND ALLOMETRIC SCALING FOR PROJECTION OF DURATION O F ACTION IN HUMANS

Table 4. Preclinical Pharmacokinetic Parameters of ZYDPLA1

Route	Mouse	Rat	Dog	Monkey
$V_{ss, iv}$ (L/kg)	7.39	8.36 ± 1.39	5.78 ± 0.99	10.49 ± 3.06
CL_{iv} (mL/min/kg)	12.39	5.64 ± 0.51	2.84 ± 0.28	3.06 ± 0.33
$T_{½, iv}$ (h)	42.21	62.82 ± 17.28	45.34 ± 0.71	71.76 ± 17.23
$T_{½, po}$ (h)	43.15	59.48 ± 6.44	28.87 ± 1.57	44.19 ± 5.66
Bioavailability (%F)	100%	100%	62 ± 11%	70 ± 6%

Intravenous bolus (iv) and oral (po) pharmacokinetic studies were performed (mg/kg doses in parentheses) in C57 mice (iv: 1; po: 3), Wistar rats (iv-1; po-2), Beagle dogs (iv-0.5; po-2 and rhesus monkeys (iv-0.5; po-2).Mean ± SEM (n=3)

Table 5. Allometric scaling indicates long half life of ZYDPLA1 in humans

Allometric Scaling Approach	Predicted human half-life (h)
Simple scaling	**53.5**
MLP-based scaling for clearance	**98.2**
Brain weight-based scaling for clearance	**152.3**

CONCLUSION

ZYDPLA1 is a novel, potent and selective oral DPP-4 inhibitor with potential to become once weekly therapy for treatment of type 2 diabetes mellitus.