ZP8396, a novel amylin analogue induces weight loss in DIO rats with a formulation space at physiological pH

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AIM

- The aim of the study was to explore formulation space of ZP8396 and to investigate in-vivo efficacy of ZP8396 formulated at physiological pH in lean, and diet-induced obese (DIO) rats

CONCLUSIONS

- **ZP8396, at concentration supporting pharmacological relevant human doses, is stable in an aqueous formulation at physiological pH and shows no aggregation potential**
- **ZP8396 is compatible with different physiological buffer systems and isotonic agents, commonly used for commercial medicinal products which provides opportunities for co-formulation with other peptides for optimal body weight loss**
- **Formulation of ZP8396 at physiological pH induces a significant body weight loss in both lean and DIO rats**

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INTRODUCTION

- Amylin acts via amylin receptors in the hindbrain to suppress appetite, which makes it a promising candidate for the development of novel pharmacotherapies for weight management.
- ZP8396, currently in phase 1, is a long-acting amylin analogue with pI of 4.36 designed to improve solubility and stability.
- ZP8396 is soluble and chemically stable in an aqueous formulation at concentrations supporting pharmacologically relevant doses.

METHODS

- Fibrilation was measured in a standard ThioflavinT (ThT) assay at 40ºC with agitation and data collection every 10 minutes for 60 hours.
- Physical stability after accelerated storage was measured by visual appearance, UV absorbance at 325nm (turbidity), Size Exclusion Chromatography (SEC, covalent oligomers), Dynamic Light Scattering (DLS, peptide particle size), or one-point ThT assay (fibrillation).
- *In vivo* efficacy was measured after single sc administration of ZP8396 (0,5, 3, 30, 300 nmol/kg) to lean rats and after 21 days of dosing with ZP8396 (1, 3, 10 and 15 nmol/kg, s.c., every 2nd day) in DIO rats. ZP8396 was formulated in 20 mM Phosphate pH 7, 260 mM mannitol buffer.

RESULTS

No signs of fibrillation of ZP8396 in aqueous formulation in a physiologically relevant pH range and no pH effect on the peptide particle size in formulation

Table 1: Lag-time in fluorescent ThT assay with physical stress at 40ºC and peptide particle size in formulation.

pH[1]	5.8	6.0	6.3	6.6	6.8	7.0
ThT lag time (h)	> 60	> 60	> 60	> 60	> 60	> 60
Peptide particle size (nm)[2]	3.3	4.0	3.2	3.0	3.7	3.0

[1] pH in TRIS buffered isotonic aqueous solution of ZP8396 (1 mg/ml).
[2] The initial peptide particle size (z-average) was evaluated by Dynamic Light Scattering (DLS).
h, hour; ThT, ThioflavinT.

RESULTS

ZP8396 at concentrations of 1-10 mg/mL is physically stable during 1 month storage at 40ºC or 3 months storage at 25ºC in formulations with different buffer systems (5-20mM), commonly used tonicity agents, and physiologically relevant pH range (6.5 - 7.4).

Table 2: Physical stability of 1-10 mg/mL ZP8396 in 5-20 mM physiological buffer systems including commonly used tonicity agents, e.g Sodium Chloride, Mannitol or Propylene glycol. Data reflects 1 month storage at 40ºC or 3 months storage at 25ºC.

Buffer system	Tested pH range	Aggregation[1]	Peptide particle size (nm)[2]	Covalent oligormers[3]
Histidine	6.5 - 7.6	Not detected	1.9 - 3.1	≤ 0.2%
Phosphate	6.1 - 7.4	Not detected	2.2 - 4.2	≤ 0.2%
TRIS	6.6 - 7.4	Not detected	2.2 - 3.5	≤ 0.3%

[1] Aggregation was evaluated by static ThT assay, UV absorbance at 325nm and/or visual appearance.
[2] The peptide particle size (z-average) was evaluated by DLS.
[3] Fraction of covalent oligomers was measured by SEC.

RESULTS

ZP8396 is physically stable during 4 weeks rotation at room temperature in 4 mg/mL aqueous formulations at a physiologically relevant pH range

Table 3: Physical stability of 4 mg/mL ZP8396 formulations rotated 4 weeks at room temperature in Type 1 glass vials. Response parameters included ThT, appearance, covalent oligomers by SEC and peptide particle size (Z-average) by DLS.

Formulation[1]	ThT assay	Appearance[2]	Covalent oligormers (SEC)	Peptide particle size (DLS)
Phosphate buffer, pH 6.1	No fibrillation[3]	Clear	< 0.2%	No increase over time (range 3.2 nm - 4.8 nm)
Phosphate buffer, pH 6.5	No fibrillation[3]	Clear	< 0.2%	No increase over time (range 3.4 nm - 5.1 nm)
TRIS buffer, pH 6.9	No fibrillation[3]	Clear	< 0.2%	No increase over time (range 3.1 nm - 4.5 nm)

[1] Tonicity controlled by commercially relevant tonicity agent, e.g. Sodium Chloride or Mannitol
[2] Appearance was evaluated visually and by UV absorbance at 325nm.
[3] No significant increase in relative fluorescent units (RFU) during study.

RESULTS

ZP8396 induces significant body weight loss in both lean and DIO rats

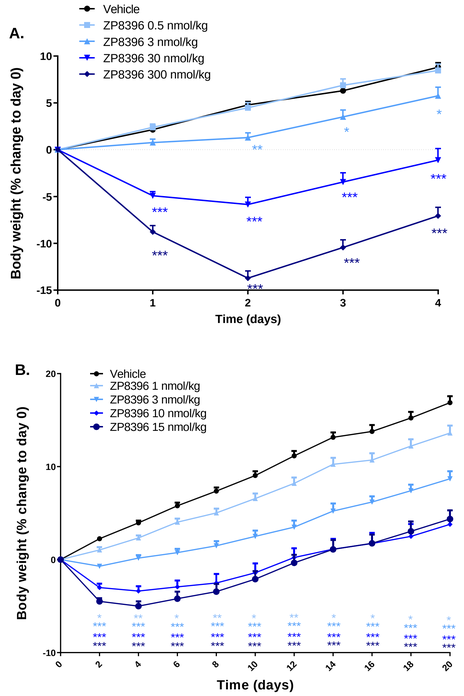

Figure 1: The effects of treatment with ZP8396 or vehicle on body weight change from day 0 (%). (A) Body weight change in lean rats, (B), Body weight change in DIO rats. Data are mean values with SEM (n= 8-10/group). Data were compared by 2-way ANOVA followed by Bonferroni multiple comparison test vs. vehicle group. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle.

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