Glucagon is used for the treatment of severe episodes of hypoglycemia in diabetic patients. Pharmacologically, however, native glucagon possesses poor physicochemical properties, making convenient administration in a ready-to-use rescue pen or the development of an artificial pancreas difficult. Accordingly the development of novel glucagon analogues such as ZP-GA-1 is pursued.

The novel glucagon analogue ZP-GA-1, which has been optimized for liquid formulation, displays enhanced solubility and improved stability at physiological pH, retaining a comparable pharmacokinetic (PK) and pharmacodynamic (PD) profile. Human glucagon receptor activation by ZP-GA-1 is comparable to native glucagon with an EC50 of 0.025 vs. 0.013 nM for glucagon (cAMP AlphaScreen assay).

**Superior physicochemical properties**

The solubility of ZP-GA-1 at physiological pH was shown to be >25 mg/mL and thus highly superior to that of native glucagon (~0.2 mg/mL). The chemical stability of ZP-GA-1 was measured over time near physiological pH and was found to be drastically improved compared to native glucagon. While glucagon showed a degradation of 51 % after 7 days at 40°C, the degradation of ZP-GA-1 after 7 days was only 1.8 %. After 360 days at 5°C the degradation of ZP-GA-1 was 3.3 % (Figure 1). These data suggest that ZP-GA-1 is suitable for long term storage as a liquid formulation.

**Comparable pharmacokinetic and pharmacodynamic profiles in the dog**

The PK and PD properties of ZP-GA-1 and native glucagon were investigated in dogs. Our data demonstrated overall similar PK profiles as well as plasma glucose profiles of ZP-GA-1 and native glucagon (Figure 2). The two compounds showed comparable PK profiles, with a desirable short time to reach maximum concentration and a short half life (Figure 4).

**Similar blood glucose profiles in a rat model of hypoglycemia**

The effect of ZP-GA-1 on blood glucose in a rat model of hypoglycemia was investigated and compared with native glucagon. Both ZP-GA-1 and native glucagon restored blood glucose to baseline levels or above in a dose-dependent manner during insulin-induced hypoglycemia (Figure 3).

**Conclusions**

The novel glucagon analogue, ZP-GA-1, displays improved physicochemical properties while maintaining similar PK and PD profiles compared to native glucagon. The results of these studies suggest ZP-GA-1 as a potential candidate for the treatment and/or prevention of severe hypoglycemia in the form of a ready-to-use rescue kit and/or in an artificial pancreas device.

**Comparable pharmacokinetics in the rat**

The PK properties of ZP-GA-1 and native glucagon were investigated in rats. The two compounds showed comparable PK profiles, with a desirable short time to reach maximum concentration and a short half life (Figure 4).

**Results**

The PK and PD properties of ZP-GA-1 and native glucagon were investigated in dogs. Our data demonstrated overall similar PK profiles as well as plasma glucose profiles of ZP-GA-1 and native glucagon (Figure 2). The two compounds showed comparable PK profiles, with a desirable short time to reach maximum concentration and a short half life (Figure 4).

**Figure 2: PK and PD profiles of ZP-GA-1 and native glucagon in male beagle dogs. Four dogs were tested in a crossover study consisting of 6 dose occasions. On each dose occasion, animals received either s.c. (20 or 120 nmol/kg) or i.v. (75 nmol/kg) administrations of either ZP-GA-1 or native glucagon. Each treatment is thus with n = 4 / group. The i.v. dose was administered as a 45 min infusion. Blood samples were collected frequently for up to 4 hours after dosing for the measurement of drug (PK) and glucose (PD) plasma concentrations.**

**Figure 3: The effect of ZP-GA-1 on blood glucose in insulin-induced hypoglycemia male Sprague-Dawley rats. The rats (n = 6 / group) were injected s.c. with Vehicle 1 (saline) or insulin (0.65 IU/kg) at t = 0 and with Vehicle 2 (PBS, pH 7.4) at t = 45 min. ZP-GA-1 or native glucagon s.c. at 40 nmol/kg. Blood samples were collected for 4 hours for the measurement of glucose concentrations. Rats were fasted 4 hours prior to the study and for the duration of the study.**

**Figure 4: Plasma concentrations of native glucagon and ZP-GA-1 in anaesthetized male Sprague-Dawley rats after s.c. (left) and i.v. (right) administration of a 300 nmol/kg bolus.**