Pharmacokinetic and Pharmacodynamic Properties of an Ultra-Concentrated, Rapid-Acting Insulin Aspart Formulation

Thomas Pieber[1], Sarah Howell[2], Leon Zakrzewski[2], Jan Jezek[2], David Gerring[2]

[1]Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria, [2]Arecor Ltd., Chesterford Research Park, Little Chesterford CB10 1XL, UK

Objectives

- **To develop a novel formulation of insulin aspart with a rapid onset of action of glucose lowering at concentrations up to 1000 U/ml**
- **In comparison to currently marketed insulin analogue products the novel formulation at 1000 U/mL must demonstrate:**

  (i) A comparable pharmacodynamic and pharmacokinetic profile (i.e. a rapid onset of action of glucose lowering)

  (ii) Non-inferior in-vitro stability

Background

There is a high unmet need for a stable, rapid-acting, ultra-concentrated insulin of up to 1000 U/mL, which will not only offer a vastly superior mealtime insulin product for people requiring >200 U/day, but is also a critical step towards the advancement of the miniaturisation of insulin delivery devices. Next-generation device technology such as wearable, continuous administration patch pumps and implants are critical future developments for people living with diabetes, improving glycaemic control and compliance, thus reducing adverse states such as hypoglycaemia and diabetic ketoacidosis, as well as overall complications and mortality.

Increasing insulin concentration is a significant formulation challenge. *In- vitro*, it leads to an increase in viscosity and impairment of stability and *in-vivo* it is known to delay the onset of action of glucose lowering (e.g., de la Peña et al. Diabetes Care, 24, 2496-2501, 2011) which is undesirable from a clinical outcomes perspective. Developing a highly concentrated rapid acting insulin product is thus impossible using the formulations of the currently marketed low concentration products.

Arestat™ technology

In order to develop the ultra-concentrated formulation of insulin aspart Arecor has applied its proprietary Arestat™ formulation technology. The technology enables protein and peptide therapeutics with superior stability and desirable bioavailability profiles. The resulting formulation of insulin aspart (1000 U/mL) is highly stable, has low viscosity and demonstrates an onset of action comparable to that achieved with 100 U/mL insulin aspart in the currently marketed formulation.

Stability

Stability was assessed using size exclusion, reverse phase and visual assessment methods based to the insulin aspart monograph. Accelerated stability was performed at 30°C over 12 weeks and demonstrated that for all chemical and physical degradation pathways the novel formulation of 1000 U/mL insulin aspart met the requirements of the monograph and had comparable or improved stability compared with the 100 U/mL Control formulation based on the currently marketed rapid acting product (Fig. 1). The accelerated study, together with longer-term study at 2-8°C (data not shown) indicates achievability of ≥2-year shelf life and 30-day in-use period.

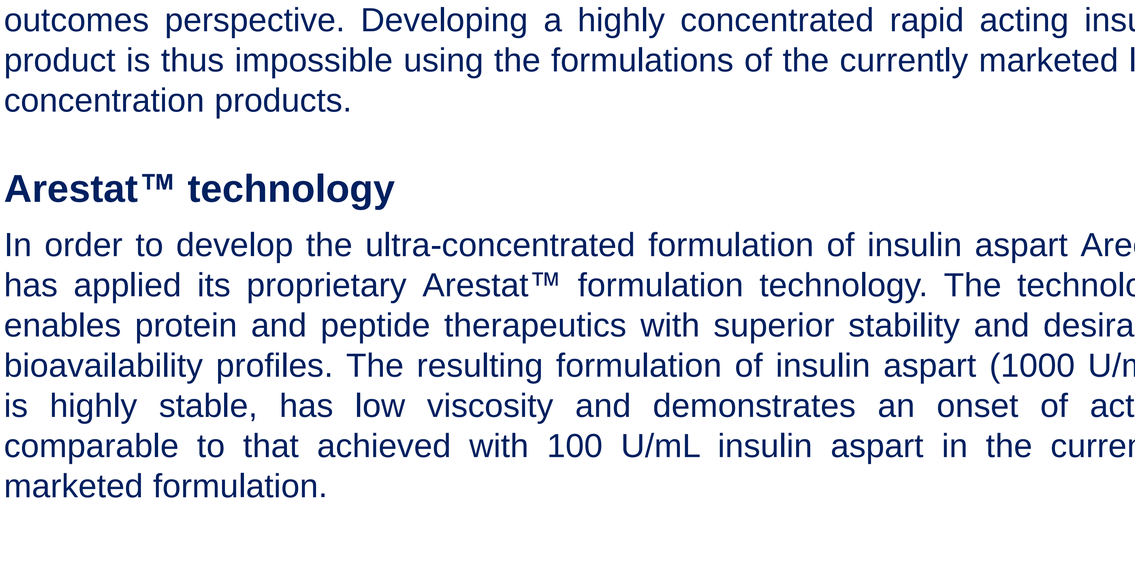

Figure 1. Stability of the novel formulation of insulin aspart (1000 U/mL) compared with Control formulation comprising 100 U/mL insulin aspart in a composition of the currently marketed product (glycerin 16 mg/mL, phenol 1.50 mg/mL, m-cresol 1.72 mg/mL, zinc 19.6 µ/mL, disodium hydrogen phosphate dihydrate 1.25 mg/mL, sodium chloride 0.58 mg/mL).

Pharmacodynamics & Pharmacokinetics

The pharmacodynamic (PD) & pharmacokinetic (PK) characteristics of the novel 1000 U/mL formulation were compared with insulin aspart Control formulations at 100 U/mL and 1000 U/mL in a diabetic mini swine, randomized, full cross-over study. Ten male animals received a dose of 0.2 U/kg insulin aspart via subcutaneous injection. Blood samples were collected at standardized intervals over a 480 min period post-injection for glucose and insulin assessment using the iso-insulin ELISA (Mercordia, AB).

Increasing the concentration of insulin aspart to 1000 U/mL in the currently marketed formulation significantly reduces the onset of action in comparison to 100 U/mL. In contrast, the novel 1000 U/mL formulation resulted in a profile comparable to that of the Control formulation at 100 U/mL (Fig. 2).

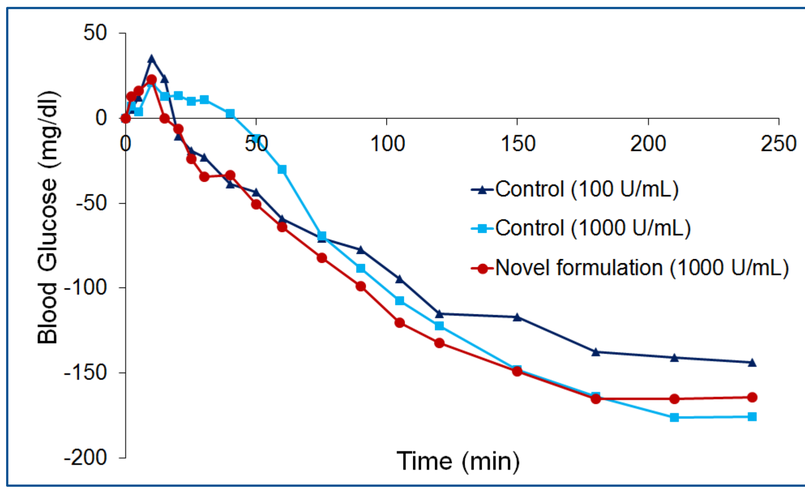

Figure 2. Baseline corrected (normalised) mean blood glucose values for the Control formulations comprising 100 U/mL and 1000 U/mL insulin aspart and the novel formulation comprising 1000 U/mL insulin aspart. See Fig. 1 for further details of the Control formulations.

The delayed onset of the 1000 U/mL Control formulation vs. the 100 U/mL control formulation was also shown in the pharmacokinetic study (T½max 28.67 min vs 8.00 min), whilst the novel 1000 U/ml formulation resulted in $T_{½max}$, T_{max} and C_{max} values that were statistically comparable (p=≤0.38) to the 100 U/mL Control. The T-½max off-rate' and the $AUC_{0\text{-}8hrs}$ were also found to be statistically comparable between 100 U/mL Control and the novel 1000 U/mL formulation (Fig. 3).

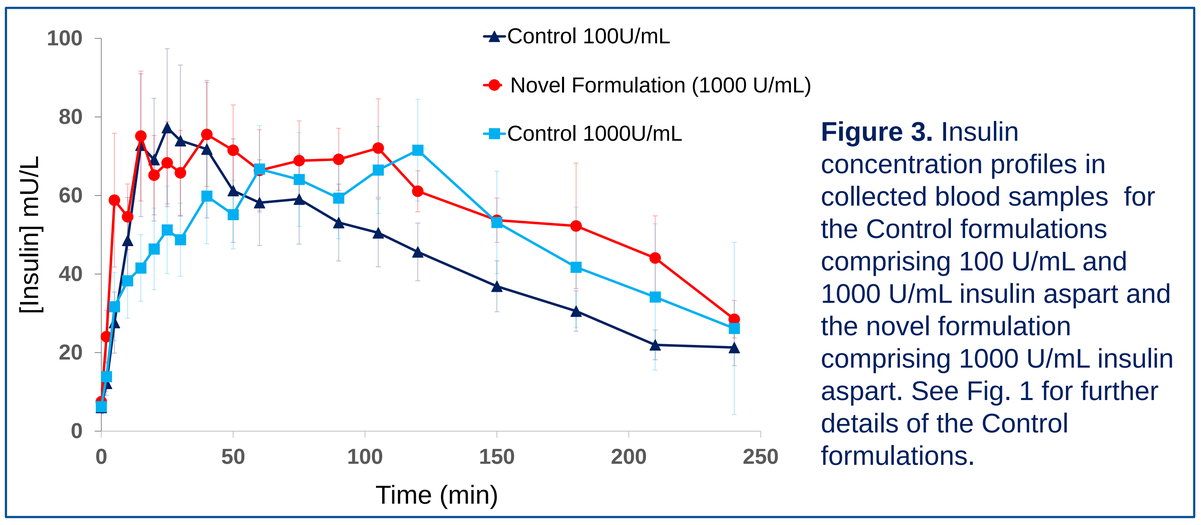

Figure 3. Insulin concentration profiles in collected blood samples for the Control formulations comprising 100 U/mL and 1000 U/mL insulin aspart and the novel formulation comprising 1000 U/mL insulin aspart. See Fig. 1 for further details of the Control formulations.

	T½max (min) Average	P-value	Tmax (min) Average	P-value	Cmax (mU/L/min) Average	P-value
Novel formulation (1000 U/mL)	7.00	-	25.71	-	90.71	-
Control (100 U/mL)	8.00	0.38	20.71	0.25	117.29	0.21
Control (1000 U/mL)	28.67	0.01	90.83	0.02	103.17	0.57

Safety

Safety of the key enabling novel excipient has been established via *in vitro* genotox and *in vivo* 7 and 28 day repeat dose toxicity studies in Wistar rats, at exposure levels up to ca. 1613 times the human equivalent dose. There were no premature decedents and no clinical observations. Body weights and food consumption were unaffected and bioanalysis indicated clearance below LLOQ after ≤4 hours.

Acknowledgement

This study was supported by JDRF. We wish to thank JDRF for their invaluable help in developing the novel formulation reported here.

Conclusions

- **Arecor used its proprietary Arestat™ technology and developed a novel formulation of insulin aspart at 1000 U/ml**
- **AT254 demonstrated stability that is comparable or better than that of the currently marketed formulation of insulin aspart at 100 U/mL as well as comparable PK/PD profile**
- **AT254 is a critical step toward advancement of miniaturisation of insulin delivery devices as well as offering a superior product for people requiring ≥200 U of insulin per day**
- **Phase 1 clinical trial in Type 1 subjects first dose planned for 2018**

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