research letter

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Investigational new insulin glargine 300 U/ml has the same metabolism as insulin glargine 100 U/ml

Insulin glargine is processed *in vivo* into soluble 21^A-Gly-human insulin (M1), the principal moiety responsible for metabolic effects, and subsequently into M2. This sub-study compared metabolism and metabolite pharmacokinetic (PK) profiles of investigational new insulin glargine U300 (Gla-300) with insulin glargine 100 U/ml (Gla-100, Lantus®, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) in people with type 1 diabetes. Participants received 0.4 (n = 18) or 0.6 U/kg Gla-300 (n = 12), and 0.4 U/kg Gla-100 (n = 30) once daily in randomized order for 8 days prior to a 36-h euglycaemic clamp. Metabolites were quantified using immunoaffinity enrichment and liquid chromatography tandem mass spectrometry (LC-MS/MS). Glargine metabolism was the same regardless of Gla-100 or Gla-300 administration; M1 was confirmed as the principal active moiety circulating in blood. Steady state concentrations of M1 were achieved after 2 days for Gla-100, and 4 days for Gla-300. Steady state M1 values defined prolonged and even flatter PK profiles after Gla-300 administration compared with M1 profiles after Gla-100.

Keywords: insulin analogues, pharmacokinetics, type 1 diabetes

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RESEARCH LETTER

Introduction

Insulin glargine 100 U/ml (Gla-100) provides a 24-h basal insulin supply after once-daily subcutaneous (SC) injection and has become a standard of care in diabetes treatment over the last decade due to its well-established efficacy and safety profiles [1]. Despite improvements in diabetes management, glycaemic control remains suboptimal in many people. A treatment to achieve tighter glycaemic control, and overcome the barriers to insulin initiation and intensification, while providing activity beyond 24 h, is required. A basal insulin supply with a further reduced peak-to-trough ratio, conferring a prolonged duration of action, could help to achieve this; investigational new insulin glargine U300 (Gla-300), comprising 300 U/ml insulin glargine, delivers insulin glargine at a reduced rate from the SC precipitate, resulting in even flatter and prolonged pharmacokinetic (PK) and pharmacodynamic (PD) profiles with longer tight glycaemic control beyond 24 h compared with Gla-100 [2].

Insulin glargine (M0; 21^A-Gly-31^B-Arg-32^B-Arg-human insulin) is a 21^A-Gly-modified mimic of 31^B-Arg-32^B-Arg-insulin, a final intermediate of natural human insulin (Figure S1) [3]. Similar to the maturation of human insulin in beta cells, after SC injection of insulin glargine (M0), enzymatic removal of the two C-terminal arginines upon re-dissolution from the SC depot yields 21^A-Gly-human insulin (M1) [4]. M1 is the predominant metabolite found in circulation, responsible for metabolic effects as demonstrated for Gla-100 in people with type 1 diabetes (T1DM) or type 2 diabetes [5,6]. Subsequent loss of threonine at position 30^B yields 21^A-Gly-des-30^B-Thr-human insulin (M2) [4]. Both M1 and M2 have a lower affinity for insulin-like growth factor 1 receptor (IGF-1R) compared with human insulin [7], and therapeutic concentrations of the parent compound and its metabolites are far below those having a mitogenic potential [5–7]. This sub-study compared the metabolism of Gla-300 with that of Gla-100 in people with T1DM.

Materials and Methods

This investigation was a sub-study of a double-blind, randomized, single-centre, 2-treatment, 2-period, 2-sequence crossover euglycaemic clamp study (ClinicalTrials.gov Identifier: NCT01349855) [2]. The study was performed in compliance with Good Clinical Practices, the Helsinki Declaration and local regulations. The protocol was approved by the ethical review board and all participants provided written informed consent.

Participants were enrolled at the Profil Institute (Neuss, Germany) and included males and females (N = 30) aged 18–65 years, with T1DM (duration ≥1 year; American Diabetes Association criteria [8]) but otherwise healthy, with HbA1c ≤9.0%, a fasting negative serum C-peptide <0.3 nmol/l and BMI 18–30 kg/m^2.

Eligible participants were randomized in two parallel cohorts (Figure S2) to receive SC once-daily doses of either 0.4 (cohort 1) U/kg or 0.6 (cohort 2) U/kg Gla-300 in one treatment period, and 0.4 U/kg Gla-100 (both cohorts) in the other, in randomized treatment order, for 8 days (at ∼20:00 hours).

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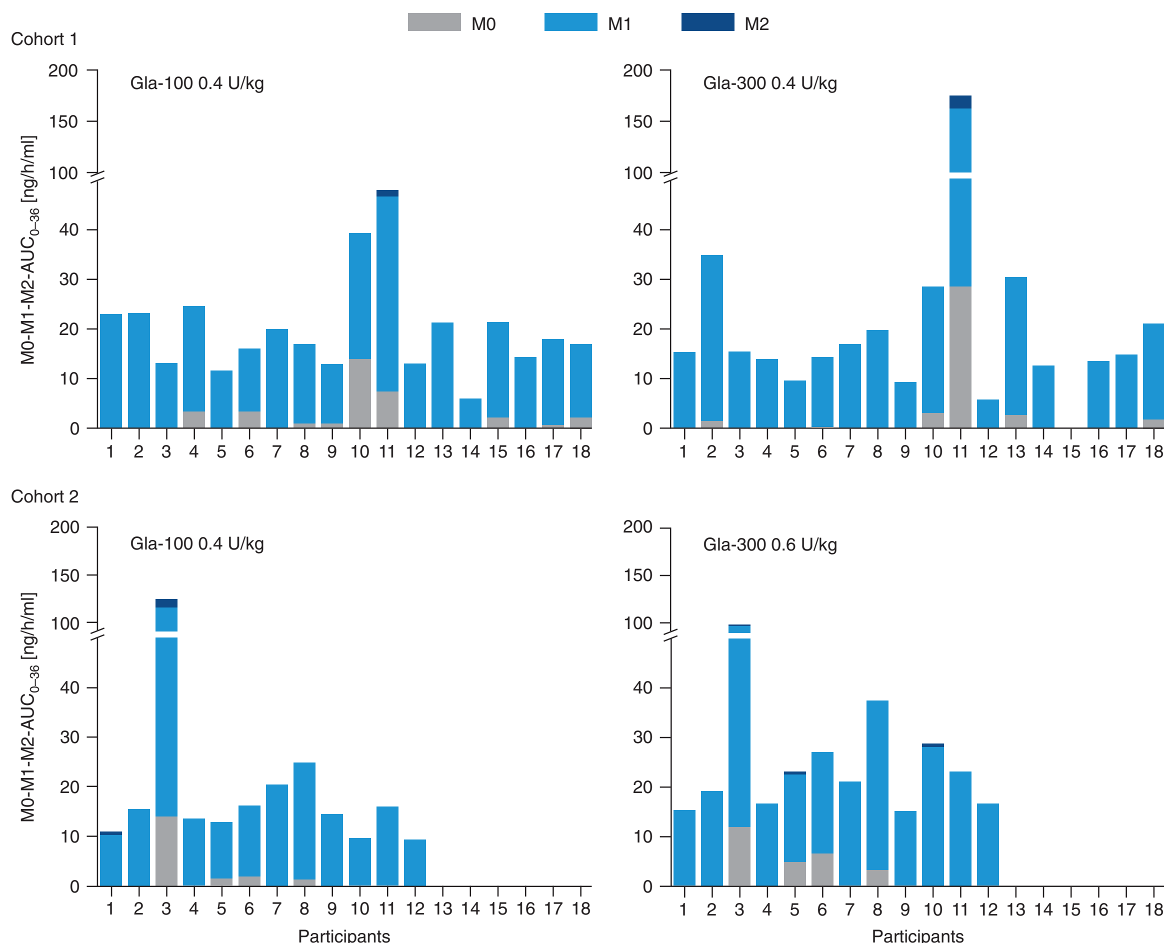

Figure 1. Cumulative exposure to M0, M1 and M2 in individual participants at steady state, assessed as the area under the insulin concentration time curve from time zero to 36 h post-dosing (M0-M1-M2-AUC_{0-36}), by treatment group.

There was a mandated washout period of 5–19 days between consecutive treatment periods. Further details regarding the study methodology have been published previously [2].

Pre-dose venous blood samples were collected to determine trough concentrations of M0, M1 and M2 on days 1–8. On day 8, a 36-h euglycaemic clamp using the Biostator™ device (MTB Medizintechnik, Amstetten, Germany) was initiated and a full PK profile was obtained. Blood samples were collected for determination of insulin concentrations at 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32 and 36 h after last dosing on day 8 (~20:00 hours).

A liquid chromatography tandem mass spectrometry (LC-MS/MS) assay with prior immunoaffinity enrichment of samples was conducted to determine M0, M1 and M2 concentrations, with a lower limit of quantification (LLOQ) of 0.2 ng/ml. Quantification of M0, M1 and M2 in plasma was unaffected by the presence of haemolysed blood (3%) or by the presence of human insulin, insulins glulisine, lispro, aspart or detemir, exenatide, liraglutide or lixisenatide at a concentration of 0.5 μg/ml.

PK parameters were evaluated by treatment using descriptive statistics. The conversion factor for concentration of plasma M1 was 1 μU/ml = 0.0344 ng/ml. Trough concentrations of M1 (C_{trough}) were plotted over time (t) by treatment, and the results of an exponential regression of the data [$C_{trough} = a(1 - \exp(-b \times t))$] – where a and b are constants (0.4 U/kg, $a = 0.603$, $b = 0.425$; 0.6 U/kg, $a = 0.723$, $b = 0.619$) – by treatment were provided.

Results

Baseline Demographics

In total, 30 participants (28 male and 2 female) with T1DM were randomized in the study. Mean age was 43.3 [standard deviation (s.d.) 8.7] years and mean BMI was 25.5 (s.d. 2.6) kg/m^2. One person dropped out prematurely due to a non-drug-related adverse event.

Concentrations of M0, M1 and M2

M1 was the principal active moiety circulating in blood after administration of both Gla-100 and Gla-300 (Figure 1).

At trough, during the first 7 days of dosing, M1 was quantifiable in almost all samples after the second or third injection, regardless of treatment and dose. Concentrations of

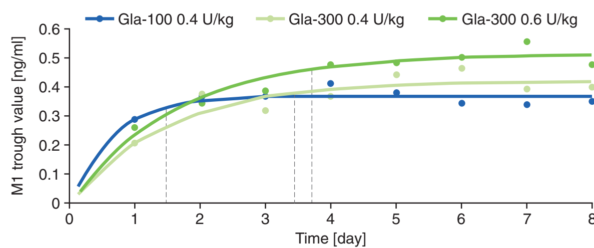

Figure 2. Median trough levels of M1 with an exponential regression of the data. Vertical dashed lines denote the time at which 90% of the plateau is achieved. For convenience, in this figure, the two Gla-100 reference groups are combined as a weighted average of the medians.

M0 and M2 were generally low and only detected in isolated samples of three and two participants, respectively. Steady state concentrations (defined as 90% of the theoretical steady state value [9]) of M1 were achieved after 2 days for Gla-100, while 4 days were required for Gla-300 (Figure 2).

At steady state, M1 was quantifiable up to 32 h for Gla-100 and 36 h (clamp end) for Gla-300 (Figure S3). In cohort 1, M0 was detected in more than two blood samples of only three participants after both Gla-100 and Gla-300 administration and in up to three further participants after either treatment. Only one participant displayed detectable M2 concentrations; this participant also displayed detectable M0 concentrations in more than two samples. In cohort 2, M0 was detected in more than two blood samples of only four participants after both Gla-100 and Gla-300 administration, one of whom also displayed detectable M2 concentrations after both treatments.

Steady State PK Profiles of M1

M1 concentration time profiles after Gla-300 administration were dose dependent and even flatter than those produced after Gla-100 administration (Figure S3). Compared with Gla-100, both Gla-300 doses were associated with lower M1 peak-to-24-h concentration differences (24-h injection interval peak-to-trough) and longer terminal half-lives (INS-$t_{1/2z}$) (Table S1). Steady state PK profiles of M1 were in line with those from unspecific radioimmunoassay (RIA) measurements [2].

Conclusions

Insulin glargine benefits from the physiology of natural human insulin formation and the retarding principle resting in the glargine molecule itself. This study demonstrates that 21^{A}-Gly-human insulin (M1) is the principal active moiety circulating in blood for both Gla-100 and Gla-300, suggesting that the metabolic effect of both is driven by M1. Steady state PK profiles of M1 after Gla-300 administration are even flatter and prolonged compared with Gla-100, in line with results from total glargine unspecific RIA measurements. Although M1 has equal glucose-lowering potency compared with parent glargine (M0) [4], *in vitro* studies demonstrate that, in contrast to M0, M1 does not exhibit an increased affinity for IGF-1R or increased mitogenicity compared with endogenous human insulin [7]. These *in vitro* data support clinical evidence from large cohort studies [10–12], in which no association between long-term treatment with Gla-100 and cancer risk was demonstrated.

In conclusion, insulin glargine metabolism in humans is the same for Gla-100 and Gla-300. In both cases 21^{A}-Gly-human insulin (M1) is the main circulating active moiety in the blood. As this metabolite has affinity for the IGF-1R similar to or lower than that of endogenous human insulin, these results support the safety profile of insulin glargine administered as either Gla-100 or Gla-300.

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Conflict of Interest

All authors are employees of Sanofi. This study was funded by Sanofi.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Metabolism of insulin glargine.
Figure S2. Study design.
Figure S3. M1 profiles at steady state.
Table S1. Pharmacokinetic parameters at steady state based on the M1 data measured with LC-MS/MS.

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