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Original Article

Insulin glargine metabolite 21^{A}-Gly-human insulin (M1) is the principal component circulating in the plasma of young children with type 1 diabetes: results from the PRESCHOOL study

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Background and Aims: Insulin glargine metabolite 21^{A}-Gly-human insulin (M1) is the principal component circulating in plasma of adults with type 1 diabetes. The objective of this study was to confirm this finding in young children and to rule out accumulation of parent insulin glargine.
Design and Methods: Children with type 1 diabetes from the PRESCHOOL study, aged 2–6 yr, were treated with insulin glargine for 24 wk (n = 62). Blood samples were drawn at weeks 1, 2, and 4 approximately 24 h after the last dose and analyzed for glargine, M1, and Thr^{30B}-des-M1 (M2) using immunoaffinity purification and liquid chromatography with mass spectrometry. The lower limit of quantification was 33 pmol/L for all analytes.
Results: M1 was the principal active component circulating in plasma. Mean (SD) plasma C_{trough} values were 101 (138), 80 (122), and 79 (102) pmol/L following glargine doses of 0.33 (0.02), 0.34 (0.02), and 0.38 (0.03) U/kg at weeks 1, 2, and 4, respectively. Parent insulin glargine and M2 concentrations were below the level of quantification. These results are in line with those observed in adults and indicate no accumulation of the parent compound in this patient population.
Conclusion: In young children with type 1 diabetes, the principal component circulating in plasma after subcutaneous injection of insulin glargine is M1, the pharmacologically active component. No accumulation of the parent insulin glargine was observed. These data provide additional evidence on the safety profile of insulin glargine in young children (Clinical trial identifier: NCT00993473).

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Key words: children – glargine metabolism – metabolite M1 – pharmacokinetic – type 1 diabetes

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The number of newly diagnosed cases of type 1 diabetes mellitus (T1DM) in young children is increasing worldwide (1). In children aged <6 yr, the management of T1DM is a challenge because the incidence of treatment-related hypoglycemia is more than double compared with older children (2, 3). The basal-bolus insulin regimen is a standard therapeutic approach in patients with T1DM (4). Furthermore, a once-daily injection of insulin glargine was recently approved by the European Medicines Agency as a treatment option in young patients with T1DM (5).

A recent multicenter, randomized, open-label, prospective study in 125 young children with T1DM (PRESCHOOL) demonstrated similar glycemic

control with once-daily insulin glargine and twice-daily neutral protamine Hagedorn (NPH) insulin (6). While glargine non-inferiority in terms of the composite endpoint was not achieved, there was only a slight difference in hypoglycemia outcomes between glargine and NPH (6). This evidence is in line with data from previous retrospective observational studies, showing that glargine leads to reduced hemoglobin A1c (HbA1c) levels as well as hypoglycemia rates compared with NPH (7, 8).

Insulin glargine was designed to mimic 31^{B}-Arg-32^{B}-Arg-human insulin, a final intermediate of endogenous insulin formation in β-cells, which despite its activity after intravenous administration, fails subcutaneously (9, 10). A single amino acid substitution, 21^{A}-asparagine for glycine (11), creates insulin glargine and renders the molecule more stable in acidic conditions causing it to precipitate amorphously. This forms a depot, from which insulin glargine is slowly released into the circulation. The result is constant basal insulin supply and 24-h duration of action (12, 13).

Subsequent enzymatic cleavage, both at the site of injection and in the circulation, leads to the formation of the main metabolite (21^{A}-Gly-human insulin, M1); further metabolism to 21^{A}-Gly-des-30^{B}-Thr (M2) is also observed (12). A recent study in adult male patients with T1DM showed that after subcutaneous injection, the exposure to glargine parent compound is marginal, even at supra-therapeutic doses, and that M1 is the principal component circulating in plasma mediating the metabolic effect (14). Although M1 has equivalent glucose-lowering potency as the parent compound (12), it exhibits lower insulin-like growth factor 1 (IGF-1) receptor affinity as well as mitogenic properties (11), compared with insulin glargine and human insulin.

There is no hint that insulin glargine pharmacokinetics (PKs) and metabolism would be different in populations of different ages, yet because no PK data are currently available on insulin glargine in young patients with T1DM, an additional objective of the PRESCHOOL study was to confirm the metabolism of glargine in young children and to rule out the potential accumulation of the parent compound. To this purpose, and as steady-state concentrations are achieved after only 1–2 daily injections, representative trough samples were taken after 1, 2, and 4 wk.

Methods

This sub-analysis of the randomized, controlled PRESCHOOL study (6) included 62 young patients (29 females) with T1DM aged 2–6 yr, who had been treated with insulin glargine (every morning) for 24 wk (Fig. 1). Of these, one patient who was randomized to NPH received insulin glargine in error. Blood samples were drawn prior to glargine injection each morning at weeks 1, 2, and 4, approximately 24 h after the last dose. In order to prevent metabolic processing in the sampled blood, venous blood was drawn into K2-EDTA vials and immediately chilled. Plasma was then obtained by centrifugation and stored at −20°C.

PK analysis of the plasma levels of insulin glargine and its metabolites was performed with C_{trough} values for insulin glargine, M1, and M2, determined using immunoaffinity and liquid chromatography tandem mass spectrometry (LC-MS/MS) (15). The lower limit of quantification (LLOQ) was 33 pmol/L for insulin glargine, M1, and M2.

The LC-MS/MS system consisted of an API 5000 triple quadruple mass spectrometer (AB SCIEX, Darmstadt, Germany) equipped with a Turbo-V-source operating in positive mode and connected to an Ultimate 3000 HPLC system (Dionex, Idstein, Germany). An inlet valve was used to truncate non-relevant signals (10-port, VICI Valco Instruments, Houston, TX, USA). For the chromatography of insulin glargine, M1, and M2, a reversed phase column was used at 40°C. A linear gradient was employed at a flow rate of 0.6 mL/min using water/formic acid (100:0.5, v/v) as mobile phase A and acetonitrile/formic acid (100:0.5, v/v) as mobile phase B. The total run time was 8.25 min and the retention times of insulin glargine, M1, and M2 were 2.07, 2.13, and 2.13 min, respectively.

Ethical approval according to local regulations was obtained from independent ethics committees and/or institutional review boards for all study sites. Conduct of the study was in line with the standards of data collection for clinical trials, according to the declaration of Helsinki. Written informed consent was obtained from the parent or legal guardian of each participant.

Statistical analyses

PK samples were to be obtained from all patients treated with insulin glargine at weeks 1, 2, and 4. According to the statistical analysis plan, to rule out accumulation of glargine, the C_{trough} value was determined for each sample approximately 24 h following the previous day's dose. Glargine concentration and metabolites M1 and M2 were determined in all samples, while only those taken at the protocol-defined sampling time were included in the statistical summary. Glargine, M1, and M2 concentrations below the LLOQ were listed as '<LLOQ'. For the statistical analysis, concentrations below LLOQ were set as 0 and included in the analysis. Under this convention, if any descriptive statistic, i.e., mean, minimum, median, or maximum, was less than the LLOQ, it was presented as '<LLOQ'; if the geometric mean was 0, it was presented as 'NC' (not computable).

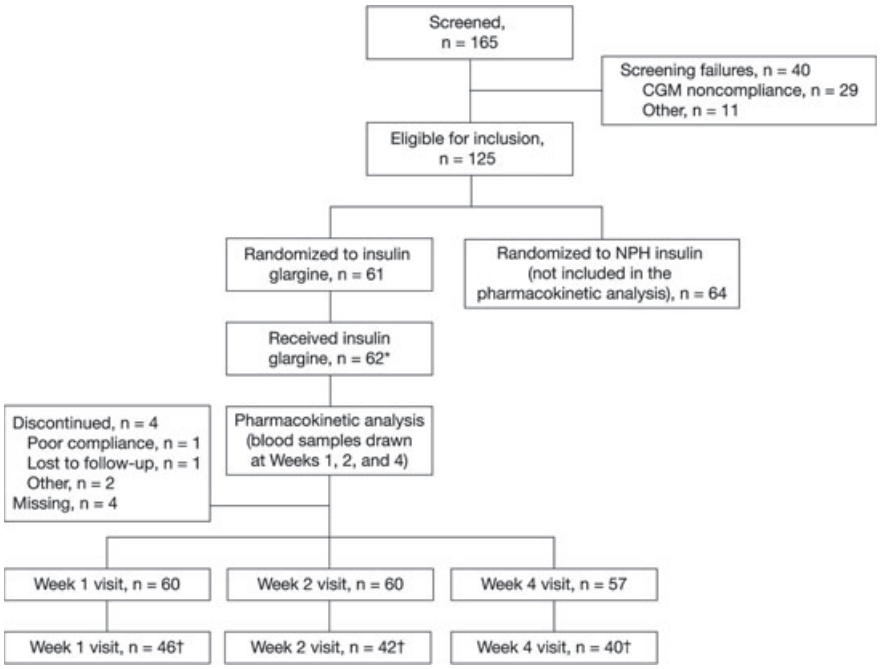

Fig. 1. Patient disposition.*One patient who was randomized to NPH insulin received insulin glargine in error; †Only those patients who met the protocol-defined sampling time for trough concentration (C_{trough}) were included in the statistical summary.

Table 1. Demographic and baseline characteristics

	DM duration, yr	HbA1c, %	HbA1c, mmol/mol	<7.5/>7.5, n (%)	Dose, U basal insulin	Dose, U/kg basal insulin	Male/ Female
Week 0 (n = 62)	2.16 (1.2) 1. 8 [1.0–5.3]	8.04 (1.05) 8.1 [6.1–10.9]	64 62 [43–96]	19 (30.6%)/ 43 (69.4%)	7.29 (4.11) 6.0 [2.0–24.0]	0.36 (0.16) 0.33 [0.1–1.0]	33/29
Week 1 (n = 46)	2.17 (1.16) 1.8 [1.0–5.3]	8.07 (0.94) 8.1 [6.2–10.5]	65 65 [44–91]	13 (28.3%)/ 33 (71.7%)	6.82 (3.09) 6.0 [2.0–16.0]	0.35 (0.12) 0.33 [0.2–0.6]	21/25
Week 2 (n = 42)	2.31 (1.17) 2.1 [1.0–5.3]	7.94 (1.02) 7.9 [6.2–10.5]	63 62 [44–91]	14 (33.3%)/ 28 (66.7%)	6.90 (3.02) 6.0 [2.0–16.0]	0.35 (0.12) 0.32 [0.2–0.6]	22/20
Week 4 (n = 40)	2.31 (1.24) 2.0 [1.0–5.3]	7.98 (1.02) 7.8 [6.2–10.5]	64 62 [44–91]	13 (32.5%)/ 27 (67.5%)	7.19 (4.08) 6.0 [2.0–24.0]	0.36 (0.16) 0.32 [0.2–1.0]	23/17

DM, diabetes mellitus; HbA1c, glycated hemoglobin.
Mean (SD); median [min–max] unless otherwise stated.

Results

Baseline characteristics

Demographics and baseline characteristics are given in Table 1. PK samples were obtained from all 62 patients treated with insulin glargine; however, eight patients (12.9%) did not have all three samples because of premature study discontinuation (n = 4) and missing samples (n = 4). Furthermore as some samples were not taken as scheduled at trough, eventually data from 46 patients at week 1, 42 at week 2, and 40 at week 4 met the predefined criteria for evaluation.

Pharmacokinetics

Insulin glargine metabolite M1 was the principal component circulating in the plasma of young children with T1DM given insulin glargine. Thirty (30) samples of 46 at week 1, 28 of 42 at week 2, and 26 of 40 at week 4 had M1 plasma concentrations >LLOQ. The mean ± SD (standard deviation) plasma M1 C_{trough} values were 101 ± 138, 80 ± 122, and 79 ± 102 pmol/L at weeks 1, 2, and 4, respectively (Table 2, Fig. 2). Only 5, 3, and 6 samples at weeks 1, 2, and 4, respectively, had parent glargine plasma concentrations >LLOQ, and 0, 2, and 1 had M2 > LLOQ. Thus, mean insulin glargine parent compound and metabolite M2 concentrations were below the level of quantification (Fig. 2).

The mean glargine dose at baseline was 0.35 U/kg and did not change substantially up to week 4. Individual M1 concentrations at trough and doses of insulin glargine did not correlate throughout the study and there was no increase in anti-glargine antibodies (data not shown).

Table 2. Pharmacokinetic data

		Plasma concentration (pmol/L)	
	Insulin glargine	M1	M2
Week 1 (n = 46)			
Mean (SD)	<LLOQ	101 (138)	<LLOQ
Median (range)	*<LLOQ (<LLOQ: 86)*	*51 (<LLOQ: 577)*	*<LLOQ (<LLOQ: <LLOQ)*
Week 2 (n = 42)			
Mean (SD)	<LLOQ (–)	80 (122)	<LLOQ (–)
Median (range)	*<LLOQ (<LLOQ: 89)*	*47 (<LLOQ: 569)*	*<LLOQ (<LLOQ: 77)*
Week 4 (n = 40)			
Mean (SD)	<LLOQ (–)	79 (102)	<LLOQ (–)
Median (range)	*<LLOQ (<LLOQ: 71)*	*0.53 (<LLOQ: 495)*	*<LLOQ (<LLOQ: 0.51)*

LLOQ, lower limit of quantification was 33 pmol/L for insulin glargine, M1, and M2; SD, standard deviation.
Italics values are median values to differentiate from the mean.

Discussion

This is the first study to assess the metabolism of insulin glargine in young children with T1DM to date. As in adults [healthy individuals and patients with T1DM/type 2 diabetes mellitus (T2DM)] (12–14), these results demonstrate that 21^{A}-Gly-human insulin (M1) is also the principal component circulating in the plasma of young children with T1DM treated with insulin glargine. After subcutaneous injection of insulin glargine, steady-state M1 plasma concentrations at trough were no different after 1, 2, and 4 weeks. In addition, our data showed that the average dose, 0.35 U/kg, corresponded to same weight-adjusted doses in adults. Vice versa, there was no positive correlation between individual M1 concentrations and absolute doses of insulin glargine, which reflects similar exposure at weight-adjusted dosing. Also, no increases

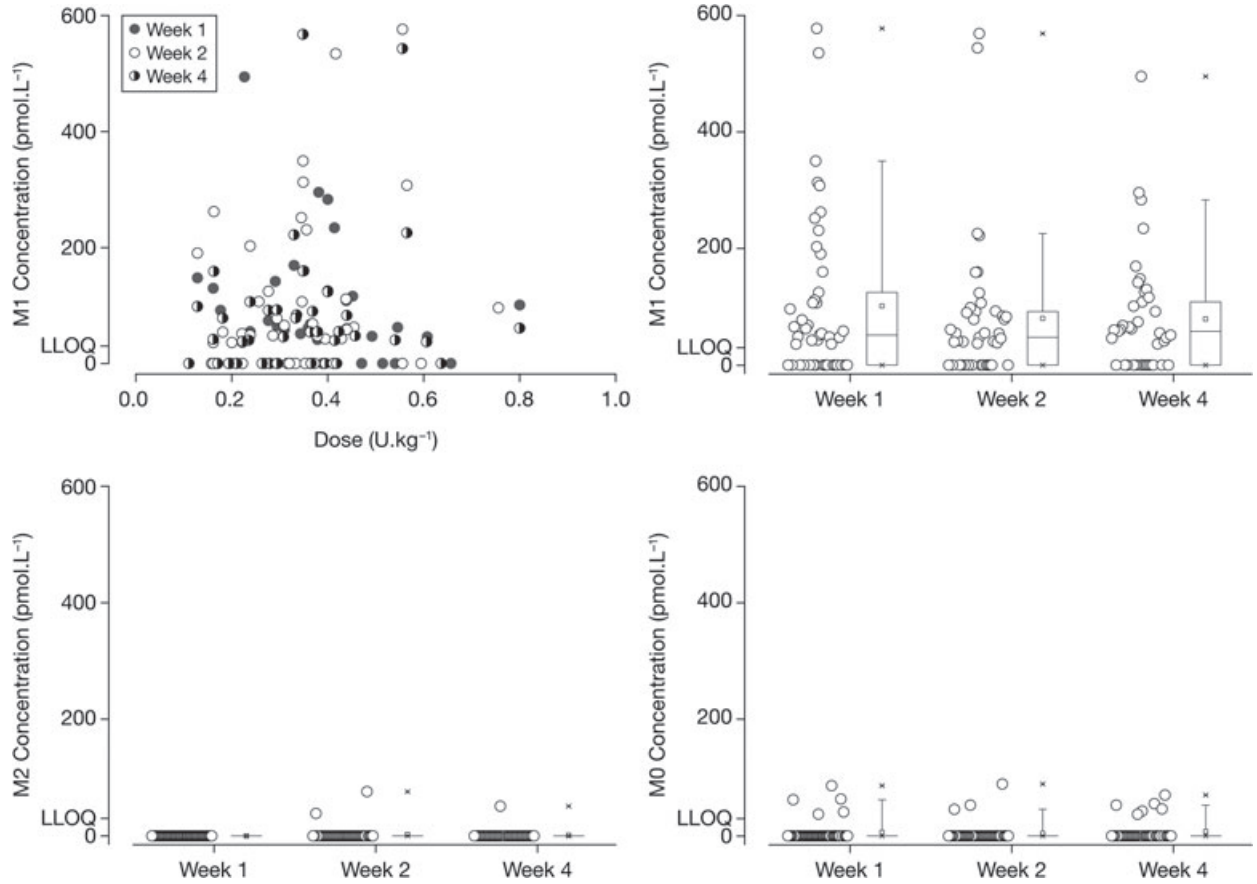

Fig. 2. Plasma concentration and correlation analysis of parent glargine, M1, and M2 metabolites in children with T1DM treated with insulin glargine after 1, 2, and 4 wk.

in anti-glargine/metabolite antibodies were observed throughout the study.

In order to avoid the burden of frequent blood sampling in a very young patient population, we investigated metabolite patterns at trough only. As such, our results, while in accordance with confirmed findings in adult patients, are limited to a certain degree. However, they clearly demonstrate that the exposure to parent compound is marginal, ruling out accumulation even at supra-therapeutic doses (13, 14). Because on average M2 levels were also below the level of detection, it was concluded that M1, and not glargine itself, mediated the glucodynamic effects (14). Therefore, absence of insulin glargine from the circulation after subcutaneous injection invalidates the hypothetical link between *in vitro* findings of enhanced IGF-1 binding and *in vivo* mitogenicity. Like its natural human insulin model 31^{B}-Arg-32^{B}-Arg-human insulin, insulin glargine is rapidly cleaved *in vivo* into its metabolite M1 and sparsely to M2, both of which have similar metabolic and lower mitogenic potencies to human insulin (11, 16).

It should be noted that in the present study only one determination of the parent compound, 24 h after subcutaneous glargine injection, was made; as such, these findings may not necessarily reflect the true parent compound values over the previous 24 h(17).

The findings concerning glargine metabolism in adult patients with T1DM and T2DM, as well as young patients with T1DM, represent a critical piece of evidence in support of the recent data from the ORIGIN study (a randomized clinical trial in more than 12 000 T2DM patients treated with insulin glargine for more than 6 yr) (18); two French cohort studies based on the French National Health Insurance Database (19, 20); and a meta-analysis of 11 studies (including 448 928 study patients and 19 128 cancer patients with diabetes) (21). In all of these studies, there was no association between long-term exposure to insulin glargine and cancer risk.

Conclusions

The metabolism of insulin glargine in young patients (2–6 yr) with T1DM is like that in adult patients (14), with no observed accumulation of the parent compound. These findings confirm that insulin glargine metabolite 21^{A}-Gly-human insulin (M1) is the principal component circulating in the plasma of young children with T1DM. On the basis of these data, the mitogenic safety profile of insulin glargine appears to be equal in young children and adults.

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Conflict of interest

T. D. received honoraria for consulting or speaking engagements from several companies involved in the diabetes field. He also received grant support for conducting studies or scientific meetings from insulin and device companies. A. P. received research support and acted as a consultant and speaker for Sanofi, Eli Lilly, and Novo Nordisk. R. B. and L. P. are employees of Sanofi.

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