Pharmacokinetics and Pharmacodynamics of Three Different Formulations of Insulin Aspart: A Randomized, Double-Blind, Crossover Study in Men With Type 1 Diabetes

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OBJECTIVE

To investigate the pharmacokinetic and pharmacodynamic properties and safety of a novel formulation of insulin aspart (AT247) versus two currently marketed insulin aspart formulations (NovoRapid [IAsp] and Fiasp [faster IAsp]).

RESEARCH DESIGN AND METHODS

This single-center, randomized, double-blind, three-period, crossover study was conducted in 19 men with type 1 diabetes, receiving single dosing of trial products (0.3 units/kg) in a random order on three visits. Pharmacokinetics and pharmacodynamics were assessed during a euglycemic clamp lasting up to 8 h.

RESULTS

Onset of insulin appearance was earlier for AT247 compared with IAsp (−12 min [95% CI −14; −8], $P = 0.0004$) and faster IAsp (−2 min [−5; −2], $P = 0.0003$). Onset of action was accelerated compared with IAsp (−23 min [−37; −15], $P = 0.0004$) and faster IAsp (−9 min [−11; −3], $P = 0.0006$). Within the first 60 min, a higher exposure was observed for AT247 compared with IAsp by the area under the curve (AUC) glucose infusion rate (GIR) from 0 to 60 min ($AUC_{Asp0–60min}$: treatment ratio vs. IAsp 2.3 [1.9; 2.9] vs. faster IAsp 1.5 [1.3; 1.8]), which was underpinned by a greater early glucose-lowering effect ($AUC_{GIR,0–60min}$: treatment ratio vs. IAsp 2.8 [2.0; 5.5] vs. faster IAsp 1.7 [1.3; 2.3]). Furthermore, an earlier offset of exposure was observed for AT247 compared with IAsp (−32 min [−58; −15], $P = 0.0015$) and faster IAsp (−27 min [−85; −15], $P = 0.0017$), while duration of the glucose-lowering effect, measured by time to late half-maximum effect, did not differ significantly.

CONCLUSIONS

AT247 exhibited an earlier insulin appearance, exposure, and offset, with corresponding enhanced early glucose-lowering effect compared with IAsp and faster IAsp. It therefore represents a promising candidate in the pursuit for second-generation prandial insulin analogs to improve postprandial glycemic control.

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Rapid-acting insulin analogs, such as insulin aspart, insulin glulisine, and insulin lispro, play a substantial role in modern clinical management of both type 1 and type 2 diabetes (1). Purposely designed to achieve faster subcutaneous absorption than regular human insulin, rapid-acting analogs provide an earlier onset and shorter duration of action and should thus improve patient convenience (administration closer to or at mealtimes), optimize postprandial glycemic control, and reduce the risk of late postprandial hypoglycemia (2–4). In line with current labeling (5–7), rapid-acting analogs have to be administered within 15 min before or up to 20 min after starting a meal. However, recent reviews indicate that an injection-meal interval of 15–20 min before a meal is still required to best meet postprandial insulin needs (8,9). For practical reasons, however, most people with diabetes use a short or no premeal interval or only inject postmeal, leading to suboptimal outcomes (10–13).

Further adjustments in pharmaceutical formulations have led to a new generation of rapid-acting insulin analogs with even more accelerated action profiles (9,14,15) to better resemble physiological prandial insulin action. Of the various initial prototypes, BioChaperone Lispro (Adocia) (16,17) is entering phase 3 trials and ultrarapid lispro (URLi or LY900014; Eli Lilly) (18,19) has recently been approved by the European Medicines Agency. Faster insulin aspart (Fiasp; Novo Nordisk, Bagsværd, Denmark) (20,21) is the first analog that has been approved in the U.S., Canada, Australia, and the European Union. It is already part of multiple daily injection regimens and insulin pump therapy in clinical practice (22,23) and has recently been applied in fully closed-loop insulin therapy (24).

A novel formulation of insulin aspart (AT247; Arecor Limited, Little Chesterford, U.K.) is currently under development and designed to provide an enhanced time-action profile after subcutaneous injection. AT247 contains a hexameric form of insulin aspart and an excipient that accelerates the rate of insulin absorption from the subcutaneous injection site. The excipient binds calcium ions, and the proposed mechanism for the accelerated absorption rate is increased tissue permeability through a transient disruption of the calcium-dependent cell adhesion in the injection site via reversible interactions with the calcium-cadherin complex at the cell surface (25). In addition, AT247 contains a stabilizing surfactant and standard preservatives (phenol and *m*-cresol).

The current study investigated the pharmacokinetic and pharmacodynamic properties as well as the safety of AT247 versus two currently marketed insulin aspart formulations (NovoRapid [IAsp] and Fiasp [faster IAsp]) in a euglycemic clamp setting in men with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Trial Design

This single-center (Medical University of Graz), randomized, double-blind, three-period, crossover phase 1 trial evaluated the pharmacokinetics, pharmacodynamics, and safety of a novel formulation of insulin aspart (AT247) compared with IAsp and faster IAsp in men with type 1 diabetes. The trial protocol was reviewed and approved by the local health authority (Austrian Federal Office for Safety in Health Care, Vienna, Austria) and by the independent ethics committee of the Medical University of Graz (No. 31-201 ex 18/19). The trial was registered at ClinicalTrials.gov (NCT03959514) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (International Conference on Harmonization). All participants were recruited based on the database of the study site and gave written informed consent before any trial-related activities were initiated.

Participants

Eligible participants were men aged 18–64 years (both inclusive), diagnosed with type 1 diabetes for at least 12 months, receiving treatment with multiple daily insulin injections or insulin pump therapy for at least 12 months with total insulin dose $<$1.2 units/kg/day and bolus insulin dose $<$0.7 units/kg/day. Participants were also required to have a BMI of 18.5–35.0 kg/m^2 (both inclusive), glycated hemoglobin (HbA_{1c}) $\leq$8.5% ($\leq$69 mmol/mol), and fasting C-peptide $\leq$0.3 nmol/L. Key exclusion criteria were clinically significant concomitant diseases, clinically significant abnormal values in clinical laboratory screening tests, current treatment with drugs that might interfere with glucose metabolism, significant history of alcoholism or drug abuse, smoking more than five cigarettes per day, and a history of severe allergies to medications or foods. Eligibility was determined on the screening visit, and eligibility for continuation was confirmed the day before trial product administration of dosing visit 1.

Procedures

The trial consisted of an informed consent visit, a screening visit, three dosing visits (separated by a 3- to 15-day washout period to minimize carryover effects), and a follow-up visit. The random allocation sequence was generated using an interactive web response system (https://www.randomizer.at) by staff with no other involvement in the trial. Permuted block randomization with a block size of six was used. On the day of the trial product administration on dosing visit 1, participants were randomly assigned with equal allocation to one of six treatment sequences (Supplementary Fig. 1). Unblinded staff, who were not involved in any other trial activities, ensured the correct treatment allocation and dispensing of trial products on the dosing visits. They transferred all trial products into 1-mL disposable syringes by using the four-eye principle. To ensure double-blinding, the trial products were then administered by blinded investigators.

On the three dosing visits, participants received a single dose of 0.3 units/kg AT247 (100 units/mL) (Arecor Limited), IAsp (NovoRapid 100 units/mL) (Novo Nordisk), or faster IAsp (Fiasp 100 units/mL) (Novo Nordisk). All trial products were administered subcutaneously into a lifted skin fold of the abdominal wall around the umbilicus. To ensure a proper basal insulin washout, participants treated with ultra-long-acting and long-acting insulin analogs were switched to NPH insulin (Insulatard; 100 units/mL in a 3-mL FlexPen) (Novo Nordisk) 72 and 48 h before trial product administration, respectively, before each dosing visit. Use of intermediate-acting and short-acting insulin products was terminated 18 and 14 h before dosing, respectively.

On each dosing visit, participants arrived at the trial site at 6:00 P.M. the day before trial product administration and were served a standardized meal. Participants started fasting at 8:00 P.M. The pharmacodynamics of trial products were assessed by the euglycemic clamp technique, with an overnight run-in period starting at 10:00 P.M. Participants using continuous subcutaneous insulin infusion had to switch off their insulin pump, and all participants received a variable intravenous infusion of human

insulin (40 units Actrapid 100 units/mL [Novo Nordisk] in 99.6 mL saline) or glucose (20%) (Fresenius Kabi, Bad Homburg, Germany) to obtain a plasma glucose (PG) clamp target level of 5.5 mmol/L (100 mg/dL). The trial product was administered between 8:00 A.M. and 10:00 A.M. on the next day after PG had stabilized for at least 1 h without glucose infusion. The rate of insulin infusion was reduced gradually during the last 15 min and completely stopped 5 min before dosing. After trial product administration and a decrease in PG by 0.3 mmol/L (5 mg/dL) a variable intravenous glucose infusion was initiated to keep PG constant at the clamp target. PG was measured in 2- to 30-min intervals throughout the clamp, and the glucose infusion rate (GIR) was recorded as required. The clamp continued for 8 h after dosing but was terminated earlier if PG was consistently >11.1 mmol/L (200 mg/dL) without glucose infusion for at least 30 min. The quality of the conducted glucose clamps (26) for each trial product is shown in Supplementary Fig. 2 and Supplementary Table 1. Blood sampling for pharmacokinetics and safety clinical laboratory evaluations as well as all additional safety assessments were performed frequently at prespecified time intervals according to the protocol.

Assessments

Blood samples were analyzed for total free serum insulin using a validated, isoinsulin ELISA (Mercodia, Uppsala, Sweden) with a lower limit of quantification (LLoQ) of 6.4 mU/L. Because this assay is 100% cross-reactive to the human insulin administered during the clamp run-in period, free serum human insulin was additionally determined in samples up to 40 min postdose using a validated, human insulin–selective ELISA (Mercodia) with an LLoQ of 3.2 mU/L. These results were used to correct the total insulin values to obtain insulin aspart data. PG concentrations during the euglycemic clamp were measured by a glucose analyzer (Super GL 2; Dr. Müller Gerätebau GmbH, Freital, Germany).

Safety assessments included physical examinations, vital signs, electrocardiograms, and clinical laboratory evaluations as well as reporting of disease-related and adverse events (AEs) such as hypoglycemia, injection site reactions, and hypersensitivity reactions. Hypoglycemic episodes were categorized by severity (severe, documented symptomatic, and asymptomatic) according to the American Diabetes Association (27).

End Points

The primary end point was the area under the curve (AUC) for GIR from 0 to 60 min ($AUC_{GIR,0–60min}$). Secondary pharmacodynamic end points included AUC for GIR of various time intervals ($AUC_{GIR,0–16min}$, $AUC_{GIR,0–30min}$, $AUC_{GIR,0–90min}$, $AUC_{GIR,0–2h}$, and $AUC_{GIR,0–8h}$), maximum GIR (GIR_{max}), time to GIR_{max} (t_{GIRmax}), time to onset of action (time from trial product administration until PG has declined by 0.3 mmol/L), and time to 50% of GIR_{max} ($t_{50\%GIRmax}$ and $t_{Late50\%GIRmax}$, where t is the first and t_{Late} is the last time point where GIR >50% of GIR_{max}). Secondary pharmacokinetic end points included AUC for serum insulin ($AUC_{Asp,0–16min}$, $AUC_{Asp,0–30min}$, $AUC_{Asp,0–60min}$, $AUC_{Asp,0–90min}$, $AUC_{Asp,0–2h}$, and $AUC_{Asp,0–8h}$), maximum insulin concentration (C_{max}), time to C_{max} (t_{max}), time to 50% of C_{max} ($t_{Early50\%Cmax}$ and $t_{Late50\%Cmax}$, where t_{Early} and t_{Late} are time to 50% of C_{max} in the early and late part of the pharmacokinetic profile, respectively), and onset of appearance and time to disappearance (time from trial product administration until the first and last time serum insulin concentration was higher than or equal to LLoQ, respectively). Pharmacokinetic end points were assessed using baseline-corrected data, where the baseline value derived from the mean of all predose values was subtracted from insulin aspart values. After baseline correction, all negative values were set to zero. Secondary end points for safety included AEs, local tolerability, vital signs variation, electrocardiogram, and laboratory safety parameters.

Statistical Analysis

Sample size calculation was performed for the primary end point ($AUC_{GIR,0–60min}$) and was based on the results of a previous trial that had evaluated the pharmacokinetics and pharmacodynamics of IAsp and faster IAsp (20). In the current trial, the mean treatment ratio (AT247-to-IAsp) was assumed to be equal to 1.31 with a coefficient of variation of 0.39. Thus, 18 completers were required to detect a treatment difference with a power of 80% (two-sided test, 5% level of significance). According to this calculation, 18 participants were planned to be randomized. To achieve 18 completers, up to 4 replacement participants could be enrolled to account for drop-outs. The replacement participants were assigned to the same treatment sequence as the drop-outs.

Statistical analyses of pharmacokinetic and pharmacodynamic end points were performed using SAS 9.4 (SAS Institute, Cary, NC) on an intention-to-treat basis including all randomized participants who had completed at least one dosing visit. Additionally, a per-protocol analysis was performed including all participants who completed all three dosing visits (data not shown). No interim analyses were conducted.

Study end points were compared between AT247 and both IAsp and faster IAsp using a linear mixed model for the log-transformed data, with treatment, treatment sequence, and visit as fixed effects and participant as a random effect. Least square means, treatment ratios (AT247-to-IAsp, AT247-to-faster IAsp) and 95% CIs were calculated based on log-transformed data and back-transformed to the original scale. If a log transformation could not be performed due to zero values, or log-transformed data deviated from normality according to the Shapiro-Wilk test, untransformed parameters were analyzed by using the Koch adaptation of the Wilcoxon rank sum test for paired comparisons. The ratios of the treatment means (AT247-to-IAsp and AT247-to-faster IAsp) and their 95% CIs were calculated post hoc for all AUC end points using the Fieller method (28). The primary analysis results for AUC end points are provided in Supplementary Table 2. All data are presented as median (25th percentile; 75th percentile) or mean ± SD if not otherwise stated. Analyses of safety end points were performed descriptively, based on all participants who received at least one dose of AT247, IAsp, or faster IAsp.

RESULTS

Participant Disposition and Demographics

Participant disposition is provided in Supplementary Fig. 1. The trial was conducted between April and August 2019. A total of 27 individuals were screened, and 19 (including 18 participants and 1 replacement participant) were randomized and received trial products. Of these, 18 participants (95%) completed the trial. One participant withdrew consent after dosing visit 2 and was therefore replaced by a participant who was allocated to the same treatment sequence as the drop-out (Supplementary Fig. 1).

All randomized participants were Caucasian and had a mean ± SD age of

Table 1—Onset, offset and overall exposure and glucose-lowering effect for a novel formulation of insulin aspart versus currently marketed insulin aspart formulations

	AT247* $n = 19$	IAsp* $n = 18$	Faster IAsp* $n = 19$	Treatment difference† (95% CI) AT247 − IAsp	*P*	Treatment difference† (95% CI) AT247 − faster IAsp	*P*
Onset of early exposure							
Onset of appearance, min	2.0 (1.0; 3.0)	13.5 (10.0; 17.0)	5.0 (4.0; 7.0)	−11.5 (−14; −8)	0.0004	−2.0 (−5; −2)	0.0003
$t_{Early50\%Cmax}$, min	12.0 (9.0; 17.0)	37.5 (30.0; 41.0)	24.0 (20.0; 28.0)	−23.5 (−31; −19)	0.0004	−12.0 (−14; −7)	0.0004
t_{max}, min	50.0 (40.0; 60.0)	90.0 (75.0; 120.0)	75.0 (65.0; 100.0)	−35.0 (−80; −15)	0.0004	−25.0 (−50; −10)	0.0032
Onset of glucose-lowering effect							
Onset of action, min	17.0 (13.0; 24.0)	37.0 (35.0; 63.0)	23.0 (22.0; 35.0)	−23.0 (−37; −15)	0.0004	−9.0 (−11; −3)	0.0006
$t_{50\%GIRmax}$, min	30.0 (25.0; 45.0)	65.0 (50.0; 90.0)	50.0 (40.0; 60.0)	−32.5 (−50; −20)	0.0004	−20.0 (−25; −5)	0.0155
t_{GIRmax}, min	95.0 (55.0; 135.0)	140.0 (110.0; 172.0)	115.0 (85.0; 150.0)	−30.0 (−55; −15)	0.0061	−30.0 (−60; 25)	0.1292
Offset of exposure and overall exposure							
$t_{Late50\%Cmax}$, min	173.0 (133.0; 223.0)	211.5 (190.0; 287.0)	221.0 (183.0; 258.0)	−32.0 (−58; −15)	0.0015	−27.0 (−85; −15)	0.0017
Time to disappearance, min	427.0 (383.0; 480.0)	462.5 (417.0; 480.0)	474.0 (420.0; 480.0)	−12.5 (−46; 0)	0.1534	−23.0 (−49; 0)	0.0241
C_{max}, mU/L‡	138.2 ± 1.5	122.0 ± 1.4	121.3 ± 1.4	1.15 (0.99; 1.33)	0.0595	1.13 (0.98; 1.31)	0.0863
Duration of glucose-lowering effect and overall glucose-lowering effect							
$t_{Late50\%GIRmax}$, min	280.0 (210.0; 290.0)	295.0 (265.0; 330.0)	290.0 (240.0; 310.0)	−22.5 (−75; 15)	0.0843	−20.0 (−60; 0)	0.2053
GIR_{max}, mg/kg/min	9.1 (5.2; 12.7)	8.0 (6.3; 11.5)	8.4 (7.5; 11.0)	0.53 (−1.82; 3)	0.7911	0.11 (−1.19; 1.43)	1.0000

*Data are presented as median (25th percentile; 75th percentile) or geometric mean ± SD. †Median treatment difference (treatment comparison calculated using the Wilcoxon rank sum test using untransformed parameters). ‡Mean treatment ratios (95% CI) are presented for C_{max} (log-transformed data analyzed by means of a mixed-effects model and results back-transformed to the original scale).

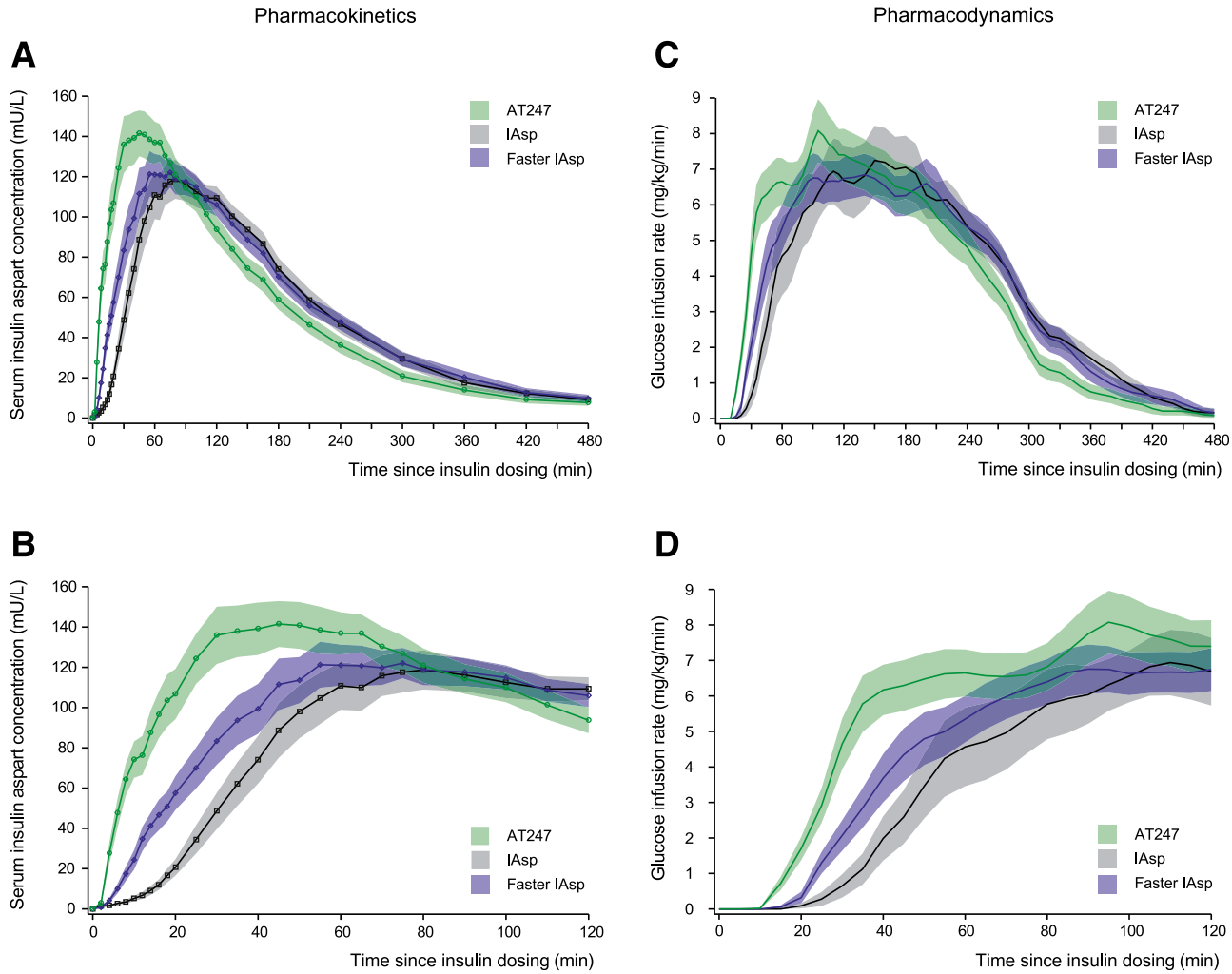

Figure 1—Pharmacokinetic and pharmacodynamic profiles after subcutaneous administration of 0.3 units/kg of a novel insulin aspart formulation (AT247), IAsp, or faster IAsp in men with type 1 diabetes. Serum insulin aspart concentration-time profiles for 8 h (*A*) and 2 h (*B*) postdose, and GIR-time profiles for 8 h (*C*) and 2 h (*D*) postdose. The GIR was averaged over 5-min intervals for the first 2 h, while 10-min intervals were used for the remaining time. Variability bands show the SEM. Number of participants: 19 for AT247 and faster IAsp; and 18 for IAsp.

35.4 ± 12.0 years. The mean body weight was 86.1 ± 13.3 kg, mean BMI was 27.2 ± 3.7 kg/m^2, mean duration of diabetes was 20.0 ± 11.1 years, mean fasting C-peptide level was 0.08 ± 0.17 ng/mL, mean HbA_{1c} was 7.1 ± 0.7% (53.8 ± 7.7 mmol/mol), and mean fasting PG was 157 ± 55 mg/dL. At entry into the trial, 7 participants were receiving multiple daily injection insulin therapy, 11 were using insulin pump therapy, and 1 was treated with insulin pump therapy in combination with bolus insulin injection.

Onset of Early Exposure and Glucose-Lowering Effect

Results on primary and secondary end points are summarized in Table 1 and Supplementary Table 3. The pharmacokinetic and pharmacodynamic profiles of all trial products are shown in Fig. 1. Early insulin exposure was significantly left-shifted for AT247 compared with IAsp and faster IAsp. Onset of appearance of AT247 occurred 2 min after administration, which was 12 and 2 min earlier than for IAsp and faster IAsp, respectively. Furthermore, $t_{Early50\%Cmax}$ and t_{max} were decreased from 38 and 90 min (IAsp) and from 24 and 75 min (faster IAsp) to 12 and 50 min when AT247 was injected. Uncorrected total insulin concentration-time profiles revealed similar differences in key end points (Supplementary Fig. 3) that did not significantly differ from insulin aspart data ($t_{Early50\%Cmax}$ and t_{max} in Table 1).

Likewise, GIR profiles were left-shifted for AT247 compared with IAsp and faster IAsp (Fig. 1), with a significant earlier onset of action (23 and 9 min earlier than for IAsp and faster IAsp, respectively). While $t_{50\%GIRmax}$ was significantly accelerated for AT247 compared with both insulin aspart reference formulations, t_{GIRmax} took place significantly earlier compared with IAsp, whereas no statistical difference compared with faster IAsp was observed.

In addition, a higher early insulin exposure and larger glucose-lowering effect up to 2 h after dosing was observed for AT247, as illustrated in Fig. 2 (data listed in Supplementary Table 3). The primary end point $AUC_{GIR,0–60min}$ was significantly enhanced for AT247 compared with IAsp and faster IAsp. Insulin exposure within the first 16 min after dosing ($AUC_{Asp,0–16min}$) was accelerated 12 and 3 times for AT247 compared with IAsp and faster IAsp, respectively. Within

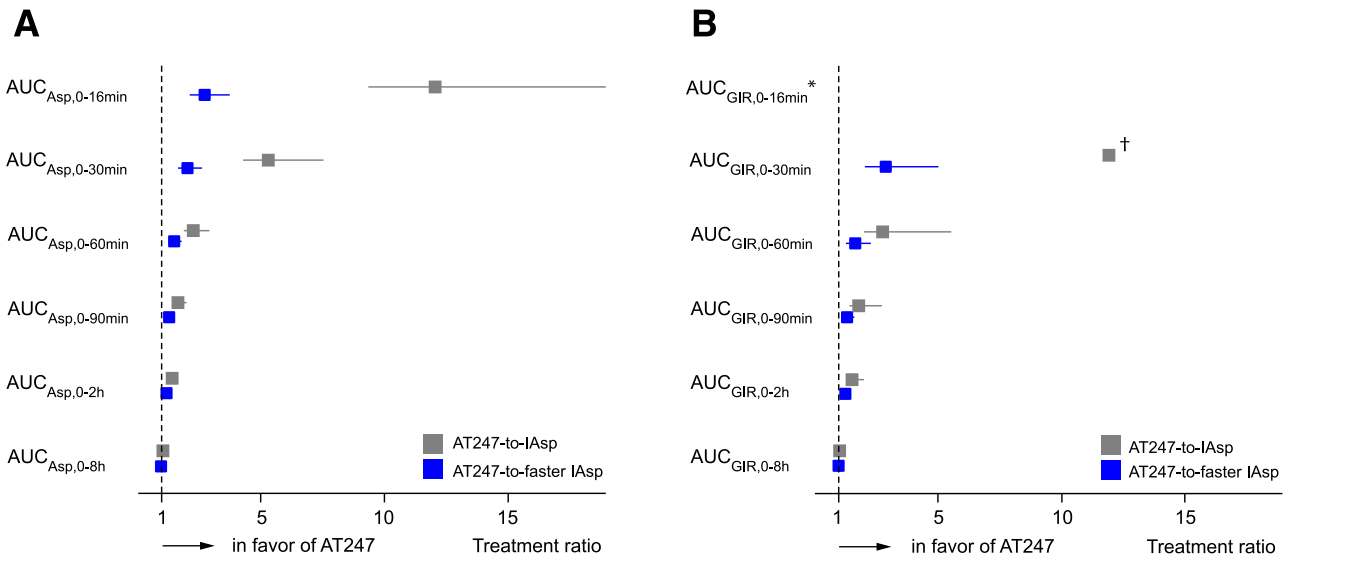

Figure 2—Exposure (*A*) and glucose-lowering effect (*B*) for a novel formulation of insulin aspart (AT247) vs. IAsp and faster IAsp. Number of participants: 19 for AT247 and faster IAsp; 18 for IAsp. Treatment ratios (95% CI) were calculated using the Fieller method (28). *Treatment ratios (AT247-to-IAsp and AT247-to-faster IAsp) were not calculable. †CI was not calculable.

the first 30 min, 5 times higher insulin exposure ($AUC_{Asp,0–30min}$) and 12 times higher glucose-lowering effect ($AUC_{GIR,0–30min}$) were observed for AT247 than for IAsp, and 2 times higher $AUC_{Asp,0–30min}$ and 3 times higher $AUC_{GIR,0–30min}$ than for faster IAsp.

Offset and Overall Exposure and Glucose-Lowering Effect

Offset of exposure, measured by $t_{Late50\%Cmax}$, occurred 32 and 27 min earlier for AT247 than for IAsp and faster IAsp, respectively. Time to disappearance of insulin aspart did not significantly differ between AT247 and IAsp but was significantly faster (23 min) compared with faster IAsp. Duration of glucose-lowering effect for AT247, measured by $t_{Late50\%GIRmax}$, was similar to both IAsp and faster IAsp (Table 1).

Moreover, overall insulin exposure ($AUC_{Asp,0–8h}$) and C_{max} were similar for all three trial products as was the overall glucose-lowering effect ($AUC_{GIR,0–8h}$) and GIR_{max} (Table 1 and Supplementary Table 3).

Safety

AT247, IAsp, and faster IAsp were well tolerated, and no clinically relevant findings were made in physical examinations, vital sign measures, electrocardiograms, and safety clinical laboratory evaluations. A total of 13 participants (68%) reported 31 AEs, which were mild to moderate in intensity and assessed as unlikely to be related to trial product administration. Only two events (nausea and injection site reaction) were considered to be treatment related, both after administration of faster IAsp. They were, however, considered mild, and the participants recovered with no action to trial product taken.

CONCLUSIONS

This is the first study to investigate the pharmacokinetics, pharmacodynamics, and safety of AT247 compared with currently marketed insulin aspart formulations (IAsp and faster IAsp) in people with type 1 diabetes. The pharmacokinetic and pharmacodynamic profiles were both left-shifted for AT247 relative to IAsp and faster IAsp profiles, and AT247 was well tolerated with no safety concerns.

Our pharmacological data within the first 2 h postdose obtained for IAsp and faster IAsp were in line with previously published individual clamp studies (20,29,30) and pooled analyses of clinical trials comparing their pharmacological properties (31–33) in type 1 diabetes. The results of the current study demonstrate that AT247 not only provides accelerated onset, exposure, and glucose-lowering properties compared with standard of care insulin aspart (IAsp) but also exceeds the fastest available prandial insulin aspart (faster IAsp). A faster absorption and earlier insulin exposure compared with IAsp and faster IAsp has recently been reported for the insulin lispro analogs BioChaperone Lispro (17) and URLi (34).

Similarly to these second-generation insulin analogs, AT247 exhibited a faster offset of exposure by a left-shift of the late part of the pharmacokinetic time profile compared with IAsp and faster IAsp. The accelerated clearance of faster IAsp versus IAsp has already been well described (31,32). These reports showed that offset of faster IAsp was 12 min earlier than for IAsp ($P < 0.001$), which is consistent with our results. Compared with faster IAsp, AT247 resulted in a significant further shortening of $t_{Late50\%Cmax}$ by 27 min. The duration of the glucose-lowering effect, measured by $t_{Late50\%GIRmax}$, has been reported to be 14 min earlier for faster IAsp versus IAsp ($P < 0.001$) (31,32). In the current study, however, the mean differences in $t_{Late50\%GIRmax}$ of 22 and 20 min comparing AT247 to IAsp and faster IAsp, respectively, were both not statistically significant. This may be attributed to the lower number of participants in the current trial. In addition, further meal challenge studies are needed to better understand the effect of an accelerated offset of exposure of AT247 in the prevention of late postprandial hypoglycemia. Similarity was observed between AT247, IAsp, and faster IAsp in overall insulin exposure and overall glucose-lowering effect. This is also in accordance with the results of the pooled pharmacological analyses of faster IAsp versus IAsp (31,32) and

confirms that the glucose-lowering potency is maintained and that only the time-concentration and time-action profiles are favorably changed.

In terms of safety, AT247 was well tolerated, and no safety concerns occurred during this single injection trial. The trial products contained insulin aspart as the active ingredient, which is considered safe based on >10 years of clinical experience (35). After a single injection of AT247, the insulin onset accelerator and stabilizer in the novel formulation did not show any negative impact on safety and tolerability; however, long-term safety needs to be confirmed.

The goal of developing second-generation insulin analogs is to overcome current difficulties and pitfalls in postprandial glycemic control. A large number of people with type 1 diabetes still struggle with correct dosing based on carbohydrate meal content, postprandial hyperglycemia, late postprandial hypoglycemia (36), timing of prandial insulin injections (13), handling of hyperglycemia corrections, or appropriate dosing for meals with a high glycemic index. Faster IAsp with its enhanced pharmacological properties partly covers these unmet needs, as shown in a range of phase 3 trials (32,35,37–40). In these trials, faster IAsp demonstrated superior postprandial glucose control accompanied with comparable or slightly improved overall glucose control and comparable risk for hypoglycemia. The even more accelerated pharmacokinetic profile of URLi has led to a further, however not statistically significant, reduction in postprandial glucose excursions compared with faster IAsp (34). Although the data on second-generation prandial insulin analogs such as AT247 are encouraging, their potential benefits for clinical practice still need to be proved (15). It is currently assumed that the benefits of faster insulin formulations will evolve from advancements in closed-loop insulin delivery, with a closed-loop algorithm adjusted to the faster action profile (24,34).

A strength of the study was its randomized, double-blind, crossover design with sufficient time to allow for insulin washout. The study population of men with type 1 diabetes was homogenous, and endogenous insulin production as a confounding factor was controlled by including C-peptide–negative participants only. The euglycemic clamp design, which is considered as the gold standard for evaluating the glucose-lowering effect of exogenous insulins (9,41), ensured comparability with other pharmacological trials (32). Based on the low coefficient of PG variability during the euglycemic clamps, the clamp quality is considered high (26) and was furthermore comparable among all trial products (Supplementary Fig. 2 and Supplementary Table 1). Although all PG values were within the clamp target range, an increase in PG was observed after insulin dosing, which was most pronounced for IAsp, followed by faster IAsp and AT247. A post hoc analysis of the data showed that this has led to an underestimation of onset of action for all three trial products, but particularly for IAsp, and consequently to an overestimation of the differences between AT247 and both reference products. However, even when considering the PG increase in the calculation of onset of action, the differences between the treatments remain significant (Supplementary Table 4).

When interpreting the results of the study, the following potential limitations have to be taken into account. There is limited generalizability of our findings to the general population of people with diabetes because the study population comprised only men with type 1 diabetes who were on average overweight. Our restriction to male participants was based on regulatory recommendations for early-phase pharmacological studies, given that insulin sensitivity in women may vary during the menstrual cycle, and whether this may affect study results is unclear (42). The study did not address dose-concentration and dose-action relationships, because only one clinically relevant dose was administered. A further limitation concerns the clamp setting, which was designed to identify potential differences between the insulin aspart formulations rather than mimic real-life conditions such as basal insulin dosing, clinically relevant and personalized bolus doses, variable dietary habits, solid mixed meals, and physical activity. To fully explore the benefits of AT247 for clinical practice, further studies are needed to assess efficacy and safety in a larger and more balanced study population and to investigate the effect on postprandial and overall glucose control in real-world settings.

In conclusion, this first-in-man study with a novel insulin aspart formulation consistently demonstrates accelerated pharmacokinetic and pharmacodynamic profiles for AT247, with earlier insulin appearance, exposure, and offset in combination with enhanced early glucose-lowering effect compared with IAsp and faster IAsp. No relevant safety findings occurred during the trial. These results suggest that AT247 represents a promising candidate in the pursuit for second-generation prandial insulin analogs to improve postprandial glycemic control in people with diabetes.

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