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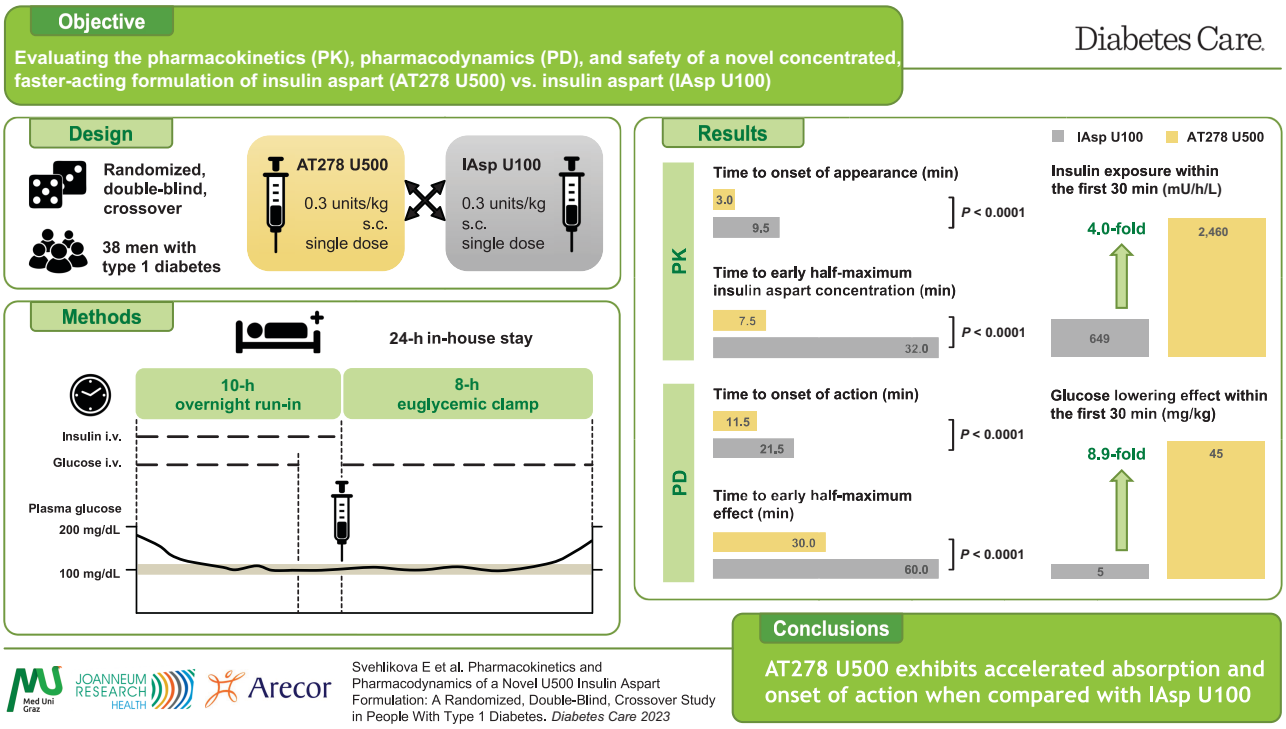

ARTICLE HIGHLIGHTS

- A novel U500 insulin aspart formulation (AT278 U500), which contains five times higher concentrated insulin aspart in a new formulation with an absorption accelerator added, for prandial coverage in severely insulin-resistant people with diabetes is currently in development.
- The aim of the current study was to evaluate pharmacokinetics, pharmacodynamics, and safety of AT278 U500.
- AT278 U500 showed accelerated absorption and onset of action when compared with U100 insulin aspart.
- AT278 U500 has the potential to improve blood glucose management and convenience for people on high-dose insulin therapy.

Pharmacokinetics and Pharmacodynamics of a Novel U500 Insulin Aspart Formulation: A Randomized, Double-Blind, Crossover Study in People With Type 1 Diabetes

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OBJECTIVE

To evaluate the pharmacokinetics, pharmacodynamics, and safety of a novel U500 insulin aspart formulation (AT278 U500) compared with insulin aspart (IAsp U100).

RESEARCH DESIGN AND METHODS

This single-center, randomized, double-blind study was conducted in 38 men with type 1 diabetes (body weight ≤100 kg and total insulin dose <1.2 units/kg/day). Participants received a single dose of either AT278 U500 or IAsp U100 (0.3 units/kg s.c.) in a crossover design, followed by an 8-h euglycemic clamp in the absence of basal insulin.

RESULTS

With AT278 U500, onset of appearance in serum was 6 min earlier ($P < 0.0001$) and reached 50% of maximum concentration 23 min faster ($P < 0.0001$). Insulin exposure with AT278 U500 was 4.0-fold higher within the first 30 min (95% CI 3.29, 4.90), 1.5-fold higher within the first 60 min (95% CI 1.35, 1.76), and statistically superior up to 90 min postdose ($P < 0.05$). With AT278 U500, onset of action was 10 min earlier ($P < 0.0001$) and reached 50% of maximum glucose infusion rate 20 min faster ($P < 0.0001$). The glucose-lowering effect with AT278 U500 was 8.9-fold higher within the first 30 min (95% CI 5.96, 17.46), 2.4-fold higher within the first 60 min (95% CI 1.92, 3.22), and statistically superior up to 2 h postdose ($P < 0.0001$). Overall insulin exposure and glucose-lowering effect were comparable. No significant safety findings were observed.

CONCLUSIONS

AT278 U500 offers rapid-acting characteristics in a reduced dose volume, with accelerated absorption and onset of action compared with IAsp U100 in the studied population.

Rapid-acting insulin analogs are today's standard in clinical care for people with diabetes who need prandial insulin replacement (1). Developed 30 years ago to more closely match physiological prandial insulin response, these analogs offer more flexibility in the timing of dosing and have demonstrated improvement of postprandial

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glucose control and a lowered risk for late postprandial hypoglycemia (2,3). More recently, reformulations of the original analogs have led to a new generation of prandial insulins with even more rapid absorption characteristics. These faster-acting insulin formulations offer additional dosing flexibility and have been shown to further improve postprandial glucose control, with a small increase in early hypoglycemia (3) and a comparable efficacy for HbA_{1c} lowering (3,4). Two faster-acting insulins have already entered the market (5,6), and a number of developments are in progress (7–10).

Prandial insulins are most commonly used in the standard concentration U100 (100 units/mL), though more concentrated preparations have become available (i.e., insulin lispro U200 [11] and insulin lispro-aabc U200 [6]). Furthermore, a U500 regular human insulin is available (12), but its action profile more closely resembles that of an intermediate-acting (NPH) insulin (1,13). Thus, it has limited convenience and efficiency for covering prandial insulin needs in severely insulin-resistant people with type 2 diabetes who require >200 units daily.

A novel concentrated, faster-acting formulation of insulin aspart (AT278 U500) is currently in development. The formulation contains 500 units/mL insulin aspart as zinc-bound hexamers and an excipient that acts as absorption accelerator by increasing the tissue permeability at the subcutaneous injection site. In addition, AT278 U500 contains a stabilizing surfactant and standard preservatives (phenol and *m*-cresol). The aim of the current study was to evaluate the pharmacokinetics (PK), pharmacodynamics (PD), and safety of AT278 U500 versus insulin aspart (IAsp U100) in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study Design

This phase 1, single-center, single-dose, randomized, double-blind, two-period crossover glucose clamp study evaluated the PK, PD, and safety of AT278 U500 (Arecor Limited, Little Chesterford, U.K.; formulation containing 500 units/mL insulin aspart sourced from Yichang HEC Changjiang Pharmaceutical Co., Ltd, Yichang, China) compared with IAsp U100 (NovoRapid; Novo Nordisk, Bagsværd, Denmark) in men with type 1 diabetes. The study 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. The study was registered at ClinicalTrials.gov (NCT04660305) and conducted in accordance with the Declaration of Helsinki and International Council for Harmonization Good Clinical Practice guidelines. All participants were recruited based on the database of the study site and gave written informed consent before any study-related activities were initiated.

Study Population

Eligible participants were men between 18 and 64 years of age (both inclusive) who were diagnosed with type 1 diabetes for at least 12 months and on 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 required to have a body weight in the range of 75–100 kg (both inclusive), glycated hemoglobin (HbA_{1c}) ≤8.5% (≤69 mmol/mol), and fasting C-peptide ≤0.3 nmol/L. Key exclusion criteria included any known or suspected hypersensitivity to the study medications or related products, clinically significant concomitant diseases, clinically significant abnormal values in clinical laboratory, vital signs, and electrocardiogram screening tests, current treatment with drugs that might interfere with glucose metabolism, significant history of alcoholism or drug abuse, and a history of severe allergies to medication or food. Eligibility was determined at the screening visit, and eligibility for continuation and dosing was confirmed at the dosing visits.

Randomization and Blinding

The random allocation sequence was generated using an interactive web response system (https://www.randomizer.at) by a third party not involved in any study activities. Randomization was performed at the day of first dosing by blinded study staff. Participants were randomly assigned with equal allocation to one of the treatment sequences and were also blinded to the treatment assignment. Unblinded staff, who were not involved in any other study activities, ensured the correct treatment allocation, preparation, and administration of study medications. The interactive web response system was programmed with blind-breaking instructions to be used by the investigator in case of an emergency. Unblinding was performed after the final database lock.

Procedures and Assessments

The study consisted of an information visit, a screening visit, two dosing visits separated by a 5- to 21-day washout period, and a follow-up visit. Participants on ultra-long-acting and long-acting insulin analogs were switched to NPH insulin (Insulatard 100 units/mL, 3 mL FlexPen; Novo Nordisk) 72 and 48 h before dosing, respectively, for basal insulin washout. The use of intermediate-acting and short-acting insulin analogs was allowed until 1000 h and 1900 h on the day before dosing, respectively. Participants on insulin pump therapy had to switch off their pump at 2200 h on the day before dosing.

Participants arrived at the study site at 1800 h on the day before dosing, were served a standardized meal, and started fasting at 2000 h. The overnight clamp run-in period started at 2200 h. 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 rate of insulin infusion was reduced gradually during the last 15 min and completely stopped 5 min before dosing. The mean PG concentrations and insulin infusion rate from 2 h predose until insulin dosing are shown in Supplementary Fig. 1. Between 0800 h and 0900 h the next day (median dosing time 0810 h), participants received a single s.c. dose of 0.3 units/kg of AT278 U500 or IAsp U100. Study medication doses were prepared gravimetrically in BD Micro-Fine 0.3 mL syringes with 30-gauge 8-mm needles (Becton Dickinson, Heidelberg, Germany) using a precision balance (CP25D-OCE; Sartorius, Göttingen, Germany) with a resolution of 0.001 mg. The doses were converted from units to dose weight by taking the specific density of the study medications into account and were drawn up according to dose weight by trained, unblinded staff. The insulin dose was administered into a lifted skin fold of the abdominal wall around the umbilicus. The dose

administered was determined by weighing the syringe before and after dosing.

PG was measured by a Super GL2 glucose analyzer (Dr. Müller Gerätebau GmbH, Freital, Germany). Time required for PG analysis (i.e., sampling, centrifugation, and measurement) was between 50 s and 1 min. PG was analyzed in 1-min intervals until values decreased by 0.3 mmol/L (5 mg/dL) relative to baseline (mean of $t = -10$, -5, and 0 min) to determine the time point when to initiate intravenous glucose infusion. For the remaining clamp duration, PG analysis and decision to adjust glucose infusion rate (GIR) was conducted in 5- to 30-min intervals to keep PG constant at the clamp target level. GIR was recorded as required. Mean PG concentration during the clamps is shown in Supplementary Fig. 2. Each clamp lasted 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. Blood samplings for PK and insulin analytics were performed as described in Supplementary Table 1. Safety laboratory evaluations were performed at prespecified time intervals according to the protocol and assessed as previously described (10).

End Points

The primary end point was noninferiority of AT278 U500 to IAsp U100 for area under the GIR time curve from 0 to 8 h ($AUC_{GIR,0–8h}$). Secondary PD end points included area under the GIR time curve of various time intervals ($AUC_{GIR,0–16min}$, $AUC_{GIR,0–30min}$, $AUC_{GIR,0–60min}$, $AUC_{GIR,0–90min}$, and $AUC_{GIR,0–2h}$), maximum GIR (GIR_{max}), time to GIR_{max} (t_{GIRmax}), time to onset of action (time after insulin dosing until PG has declined by 0.3 mmol/L), and time to 50% of GIR_{max} ($t_{Early50\%GIRmax}$ and $t_{Late50\%GIRmax}$, where t_{Early} is the first and t_{Late} is the last time point at which GIR >50% of GIR_{max}). Secondary PK end points were defined and derived as described in Supplementary Table 1. Safety end points were defined and derived as previously described (10).

Statistical Analysis

Sample size calculation was based on comparison of the primary end point $AUC_{GIR,0–8h}$ assuming a mean treatment ratio ± SD of 0.95 ± 0.35. Comparison of the secondary end point $AUC_{GIR,0–60min}$, assuming a mean treatment difference ± SD of 0.325 ± 0.68 on log-scale, was additionally taken into consideration. Assumptions were based on data from a previous study comparing AT247, IAsp U100, and faster insulin aspart in men with type 1 diabetes (10). A total of 28 completers were required to show noninferiority of AT278 U500 for $AUC_{GIR,0–8h}$ with 80% power (one-sided test, 5% level of significance) and 37 completers to show superiority of AT278 U500 for $AUC_{GIR,0–60min}$ with 80% power (two-sided test, 5% level of significance). Overall, 38 participants were planned to be randomly assigned to avoid underpowering due to uncertainties in the estimation of mean and SD used in the power calculation.

Statistical analyses of end points were performed using SAS 9.4 (SAS Institute, Cary, NC) on an intention-to-treat basis including all randomly assigned participants who had completed at least one dosing visit. No interim analyses were conducted. PK time variables (i.e., onset of appearance, time to maximum insulin aspart 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 PK profile, respectively], and time to disappearance) were derived from unsmoothed insulin aspart data. The PD variable time to onset of action was calculated via two different methods: *1*) as time after insulin dosing until PG has declined by 0.3 mmol/L (5 mg/dL) from baseline (data shown in Supplementary Table 2); and *2*) as time after insulin dosing until PG has declined by 0.3 mmol/L (5 mg/dL) from highest PG level measured postdose (data reported in the main article). The first method represents the standard approach, while the second method is preferred as it accounts for a potential confounding effect of a postdose PG increase. This increase is observed when insulins under investigation show a significant difference in the onset of action; thus, the standard method leads to underestimation of the onset of the slower insulin and overestimation of the difference between insulins (10).

End points are presented as median (25th percentile, 75th percentile). End points were compared between AT278 U500 and IAsp U100 (treatment ratios or treatment differences [95% CI]) using log-transformed data (AUC_{GIR}, GIR_{max}, area under the insulin aspart concentration time curve [$AUC_{Insulin}$], and C_{max} when the data followed a log-normal distribution) or untransformed data (all time variables, AUC_{GIR}, GIR_{max}, $AUC_{Insulin}$, and C_{max} when log-transformed data deviated from normality or when log-transformation could not be performed due to zero values). Log-transformed data were tested for normality using a Shapiro-Wilk test (10% level of significance), and untransformed data were tested for normality of treatment differences. Depending on data characteristics (distribution and presence of zero values), appropriate tests were applied (i.e., Student *t* test, Koch adaption of the Wilcoxon-Mann-Whitney rank sum test, or Wilcoxon signed rank test). *P* values were provided for the one-sided tests for noninferiority (noninferiority margin 0.8) and superiority of AT278 U500 and for the two-sided tests for treatment differences between the study medications. The significance level was set to 5%. In addition, 95% CIs for treatment comparisons of AUC_{GIR} variables were calculated using Fieller's theorem (14).

RESULTS

Participant Disposition and Baseline Demographics

The study was conducted between December 2020 and June 2021. A total of 49 individuals were screened. Out of these, 38 participants were included, randomly assigned, and completed the study (Supplementary Fig. 3).

The randomly assigned participants were all male, were White, and had a mean ± SD age of 38.8 ± 10.8 years. The mean body weight was 86.4 ± 8.4 kg, mean BMI was 26.9 ± 2.7 kg/m^2, mean duration of diabetes was 19.3 ± 10.8 years, median (range) fasting C-peptide level was 0.00 (0.00–0.84) ng/mL, mean HbA_{1c} was 7.0 ± 0.8% (52.8 ± 8.6 mmol/mol), and mean fasting PG was 7.8 ± 2.2 mmol/L (140.8 ± 38.7 mg/dL). At screening, 16 participants were on multiple daily injection insulin therapy and 22 participants were insulin pump users (Supplementary Table 3).

PK

The PK profile of AT278 U500 and IAsp U100 is shown in Fig. 1*A* and *B*. All PK end points for initial insulin exposure were significantly earlier for AT278 U500 compared with IAsp U100 (Table 1), with a 6-min faster insulin appearance

($P < 0.0001$), 23-min faster $t_{Early50\%Cmax}$ ($P < 0.0001$), and 44-min faster t_{max} ($P < 0.0001$). Offset of insulin exposure and overall insulin exposure, measured by $t_{Late50\%Cmax}$, time to disappearance, and C_{max}, were comparable between both insulins (Table 1), with AT278 U500 showing noninferiority ($P = 0.0127$) but no statistical superiority ($P = 0.9633$) to IAsp U100 for C_{max}. All estimates of insulin exposure with AT278 U500, as measured by $AUC_{Insulin}$, were noninferior to IAsp U100 (Table 2) (all $P < 0.0001$). Up to 90 min postdose, all $AUC_{Insulin}$ estimates with AT278 U500 were statistically superior (Table 2) ($P < 0.05$). AT278 U500 exhibited a 4.0-fold higher $AUC_{Insulin}$ within the first 30 min and a 1.5-fold higher $AUC_{Insulin}$ within the first 60 min compared with IAsp U100 (Table 2). The full $AUC_{Insulin}$ data are listed in Supplementary Table 4.

PD

The PD profile of AT278 U500 and IAsp U100 is shown in Fig. 1*C* and *D*. The onset of glucose-lowering effect was significantly earlier for AT278 U500 compared with IAsp U100 (Table 1), with a 10-min earlier onset of action ($P < 0.0001$) and a 20-min faster $t_{Early50\%GIRmax}$ ($P < 0.0001$), whereas no difference between both insulins was observed for t_{GIRmax} ($P = 0.6029$). Duration of glucose-lowering effect and overall glucose-lowering effect, measured by $t_{Late50\%GIRmax}$ and GIR_{max}, were comparable between both insulins (Table 1), with AT278 U500 showing noninferiority ($P < 0.0001$) but no statistical superiority ($P = 0.3990$) to IAsp U100 for GIR_{max}. All estimates of glucose-lowering effect with AT278 U500, as measured by AUC_{GIR}, were noninferior to IAsp U100 (Table 2) (all $P < 0.0001$), including the primary end point $AUC_{GIR,0–8h}$. Up to 2 h postdose, all AUC_{GIR} estimates with AT278 U500 were statistically superior (Table 2) ($P < 0.0001$). AT278 U500 exhibited an 8.9-fold higher AUC_{GIR} within the first 30 min and a 2.4-fold higher AUC_{GIR} within the first 60 min compared with IAsp U100 (Table 2). The full AUC_{GIR} data are listed in Supplementary Table 4.

Safety

Both AT278 U500 and IAsp U100 were well tolerated, and no safety issues were identified. A total of 32 adverse events were reported in the study, which were all mild in intensity. Of these, six adverse events were considered treatment-emergent (three with AT278 U500 and three with IAsp U100). Only one adverse event (one case of injection-site reaction) that occurred after dosing of AT278 U500 was considered related to the study medication. The injection-site reaction (burning pain right after dosing, without exanthema) was 2 cm in diameter and lasted for 10 min. The participant recovered with no medical action taken. There were no serious adverse events and no clinically significant findings in electrocardiogram, vital signs, and safety laboratory assessments reported during the study.

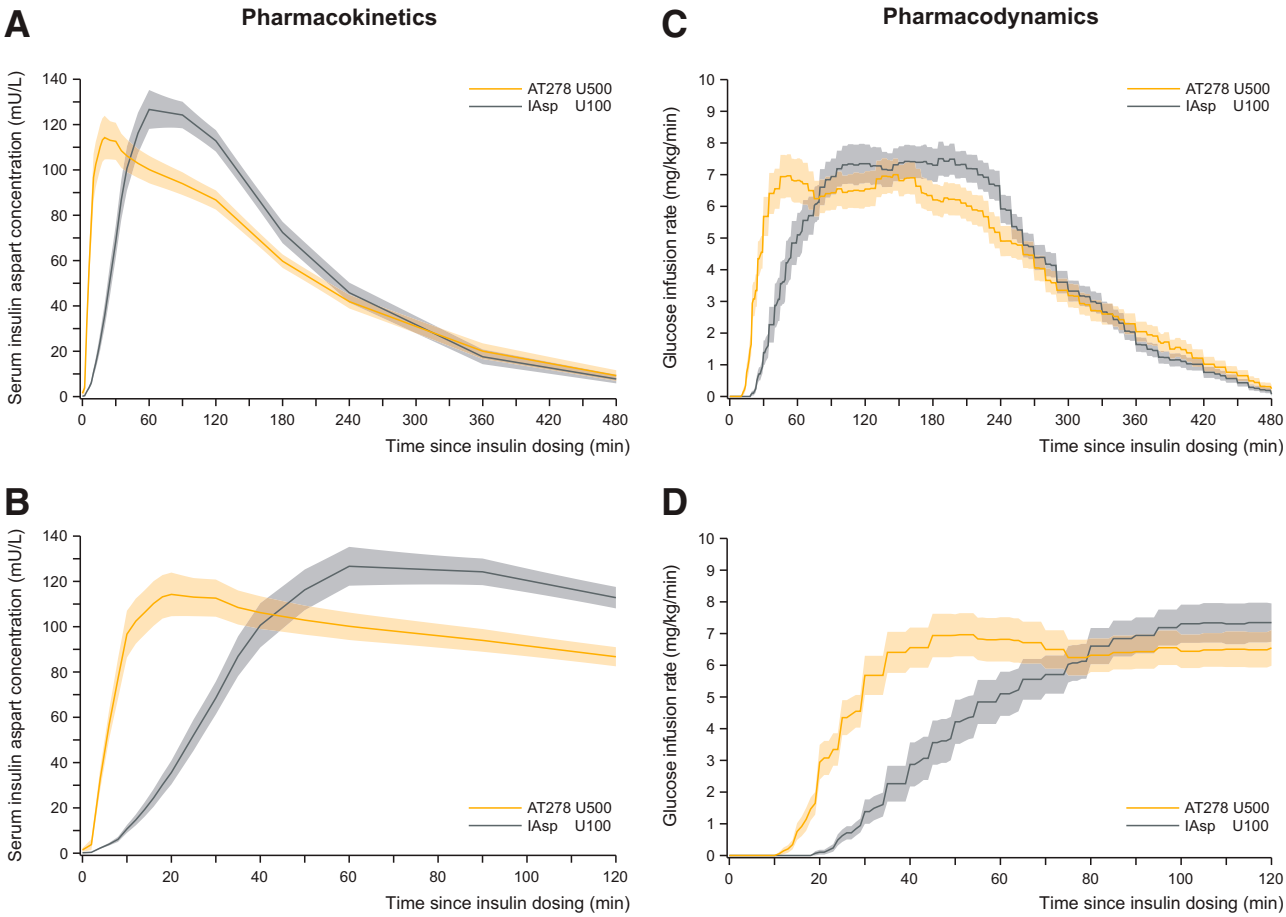

Figure 1—PK and PD of a novel U500 insulin aspart formulation (AT278 U500; orange line) and insulin aspart (IAsp U100; gray line) after s.c. administration of 0.3 units/kg in men with type 1 diabetes. Mean serum insulin aspart concentration-time profiles for 8 h (*A*) and 2 h (*B*) postdose and mean GIR-time profiles for 8 h (*C*) and 2 h (*D*) postdose. Variability bands show the SEM. Number of participants was 38 for both study medications.

Table 1—PK and PD of a novel U500 insulin aspart formulation (AT278 U500) vs. insulin aspart (IAsp U100) in men with type 1 diabetes

	AT278 U500* (n = 38)	IAsp U100* (n = 38)	Treatment difference (95% CI),* AT278 U500 − IAsp U100	P
Onset of insulin exposure				
Onset of appearance, min	3.0 (3.0, 4.0)	9.5 (8.0, 14.0)	−6.0 (−8.0, −6.0)	<0.0001
$t_{Early50\%Cmax}$, min	7.5 (6.0, 10.0)	32.0 (27.0, 35.0)	−23.0 (−26.0, −22.0)	<0.0001
t_{max}, min	25.0 (16.0, 50.0)	90.0 (60.0, 96.0)	−43.9 (−56.0, −31.8)†	<0.0001
Onset of glucose-lowering effect				
Onset of action, min	11.5 (9.0, 16.0)	21.5 (14.0, 27.0)	−9.5 (−13.0, −6.0)	<0.0001
$t_{Early50\%GIRmax}$, min	30.0 (25.0, 45.0)	60.0 (45.0, 75.0)	−20.0 (−30.0, −15.0)	<0.0001
t_{GIRmax}, min	132.5 (65.0, 180.0)	147.5 (100.0, 180.0)	−8.6 (−41.9, 24.7)†	0.6029
Offset of insulin exposure and overall insulin exposure				
$t_{Late50\%Cmax}$, min	196.0 (145.0, 277.0)	207.5 (162.0, 273.0)	2.2 (−23.8, 28.2)†	0.8639
Time to disappearance, min	443.0 (352.0, 480.0)	447.0 (349.0, 480.0)	−0.5 (−18.4, 17.5)†	0.9599
C_{max}, mU/L	116.9 (84.0, 169.0)	130.4 (101.2, 166.9)	0.91 (0.81, 1.01)‡	nc
Duration of glucose-lowering effect and overall glucose-lowering effect				
$t_{Late50\%GIRmax}$, min	304.0 (229.0, 374.0)	304.0 (249.0, 349.0)	−4.0 (−25.5, 17.6)†	0.7119
GIR_{max}, mg/kg/min	8.1 (6.5, 11.2)	8.8 (6.4, 11.1)	1.01 (0.92, 1.12)‡	nc

nc, not calculated. *Data are presented as median (25th percentile, 75th percentile) and median treatment differences (95% CI), with P value calculated by using Koch's adaptation of the Wilcoxon rank sum test. †Arithmetic mean treatment difference (95% CI), with P value calculated by using Student t test. ‡Geometric mean of treatment ratios (95% CI).

CONCLUSIONS

This is the first study evaluating PK, PD, and safety of AT278 U500 in people with type 1 diabetes. The PK profile of AT278 U500 was left-shifted in the early part of the insulin concentration time curve with comparable offset and overall insulin exposure when compared with IAsp U100. This observation was mirrored by the PD response that showed an earlier onset of action and faster $t_{Early50\%GIRmax}$ for AT278 U500, while duration of action and overall glucose-lowering effect did not significantly differ.

We assume that the specific PK profile observed with AT278 U500 is caused by two factors that inversely affect insulin absorption. AT278 U500 contains an excipient with binding sites for divalent metal cations that does not cause a substantial dissociation of the zinc-bound hexamers

Table 2—Insulin exposure and glucose-lowering effect for a novel U500 insulin aspart formulation (AT278 U500) vs. insulin aspart (IAsp U100) in men with type 1 diabetes

	Main analysis, treatment ratio (95% CI),* AT278 U500-to-IAsp U100	P		Supplementary analysis, treatment ratio (95% CI),† AT278 U500-to-IAsp U100
		Noninferiority	Superiority	
Insulin exposure, mU·h/L				
$AUC_{Insulin,0–16min}$	9.57 (6.80, 13.68)	<0.0001	<0.0001	nc
$AUC_{Insulin,0–30min}$	4.02 (3.29, 4.90)‡	<0.0001	<0.0001	nc
$AUC_{Insulin,0–60min}$	1.54 (1.35, 1.76)‡	<0.0001	<0.0001	nc
$AUC_{Insulin,0–90min}$	1.14 (1.03, 1.26)‡	<0.0001	0.0077	nc
$AUC_{Insulin,0–2h}$	1.01 (0.92, 1.10)‡	<0.0001	0.4534	nc
$AUC_{Insulin,0–8h}$	0.98 (0.92, 1.00)	<0.0001	0.9940	nc
Glucose-lowering effect, mg/kg				
$AUC_{GIR,0–16min}$	nc	nc	nc	nc
$AUC_{GIR,0–30min}$	nc	nc	nc	8.91 (5.96, 17.46)
$AUC_{GIR,0–60min}$	nc	nc	nc	2.36 (1.92, 3.22)
$AUC_{GIR,0–90min}$	1.80 (1.51, 2.16)‡	<0.0001	<0.0001	1.55 (1.37, 1.81)
$AUC_{GIR,0–2h}$	1.36 (1.18, 1.57)‡	<0.0001	<0.0001	1.26 (1.13, 1.44)
$AUC_{GIR,0–8h}$	1.02 (0.95, 1.09)‡	<0.0001	0.3089	1.01 (0.94, 1.09)

Number of participants was 38 for both study medications. nc, not calculable or not calculated. *Median of treatment ratios (95% CI). P values for noninferiority and superiority are calculated from median treatment ratios derived from untransformed data by using a Wilcoxon signed rank test (one-sided test, noninferiority margin 0.8). †Treatment ratios of arithmetic means (95% CI) calculated using Fieller's theorem. ‡Geometric mean of treatment ratios (95% CI). P values for noninferiority and superiority are calculated from arithmetic mean treatment differences of log-transformed data by using Student t test (one-sided test, noninferiority margin ln0.8). Results were back-transformed to the original scale.

into dimers/monomers but increases the tissue permeability through a transient disruption of the calcium-dependent cell adhesion via reversible interactions with the calcium–cadherin complex (15). This leads to a faster insulin absorption from the subcutaneous injection depot and consequently to a left shift in PK/PD, as seen with AT247, a U100 insulin aspart formulation containing the same excipient (10). In contrast, the fivefold higher concentration of U500 insulins is expected to reduce the rate of hexamer dissociation into dimers and monomers and to reduce the ratio of the diffusion area to the amount of insulin to be absorbed (16–18). This leads to a slower absorption and consequently to a right shift in PK/PD, as observed with regular human insulin U500 (19).

However, it seems that this concentration-dependent right shift is more pronounced with human insulins than with the analogs (20). It has been shown that both other concentrated prandial insulin analogs (i.e., insulin lispro U200 and insulin lispro-aabc U200) are bioequivalent to their respective U100 formulation (21,22). It is not yet fully understood which factor mainly counteracts the concentration-dependent right shift. It can be either the altered amino-acid sequence of insulin lispro leading to a more rapid dissociation of insulin hexamers into dimers and monomers in the injection depot, or it can be the adjustment of excipients (i.e., changing the zinc content and buffering agent), or it can be the lesser concentration of the U200 preparation compared with regular human insulin U500 (17).

In the current study, we observed a similar phenomenon with AT278 U500 (i.e., that the accelerating effect of the excipient on insulin absorption prevailed over the delaying effect of the higher concentration). The shape of the GIR curve of AT278 U500 suggests that this phenomenon is more pronounced in the early phase up to 60 min postdose (Fig. 1*C* and *D*). The initial steep rise is followed by an "undulating plateau" between 60 min and 3 h postdose rather than by a distinct peak. This could indicate that the delaying effect of the higher concentration comes into play from 60 min postdose. However, with AT278 U500, there was no delayed or lowered C_{max}, and consequently, GIR_{max} as reported for regular human insulin U500 (19). Overall insulin exposure and overall glucose-lowering effect, measured by $AUC_{Insulin,0–8h}$ and $AUC_{GIR,0–8h}$, were similar for AT278 U500 and IAsp U100, which demonstrates that the different size of the injection depots had no effect on the overall extent of insulin absorption and action. While a working hypothesis exists regarding the molecular interactions of the key excipient in AT278 U500 that cause the faster insulin absorption, the exact underlying mechanisms that lead to the specific PK/PD profile of AT278 U500 remain unclear and need further investigation to better interpret their clinical implications.

Regular human insulin U500 is the only highly concentrated insulin that is currently available. A clinically meaningful decrease in HbA_{1c} has been demonstrated in retrospective analysis of real-world data (23–26). This was attributed to the easier titration to the needed dose without the need for dose splitting, which in turn improved treatment adherence. Indeed, regular human insulin U500 is considered to target basal as well as prandial insulin needs but has to be taken 30 min before a meal due to its delayed onset (13,27), which is inconvenient in most people's daily lives and may not be followed. Moreover, recent findings suggest that the injection–meal interval might even have to be extended for high doses (i.e., 200-unit dose), concluding that regular human insulin U500 should not be used for prandial bolus dosing in severely insulin-resistant people with type 2 diabetes (28).

Given the fact that the other concentrated prandial insulin analogs do not primarily target severely insulin-resistant patients, but were developed to provide longer-lasting pens (29), availability of a U500 insulin with a rapid PK/PD profile is currently lacking. The superior insulin exposure and action of AT278 U500 within the first 30 and 60 min postdose suggest that AT278 U500 will enable dosing at meal or even after meal without compromising postprandial glucose control and will thus provide more flexibility with meals and daily schedule for people with high insulin needs. Although none of the concentrated insulins are approved for insulin pump therapy yet, several studies have reported the safe, effective, and more convenient use of concentrated insulins in insulin pumps (30–32). As more highly concentrated insulins become available, it is anticipated that smaller pumps will follow, which could further relieve the daily burden of managing diabetes.

Strengths of the current study are that it was designed in accordance with regulatory guidance for the development of biosimilar insulins (33) by using a single-dose, crossover, double-blind, euglycemic clamp design. The double-blinding allowed for reducing potential investigator-related bias regarding the manual adjustment of the GIR. The quality of the performance of the clamp study was evaluated by calculating the coefficient of variation of PG measurements and the mean difference between PG measurements and the target glucose level (control deviation) (33,34). Clamp quality results were within the range achieved with automated clamp devices (34) and comparable between the study medications (Supplementary Table 5). The large sample size further strengthened the robustness of the study.

Despite these strengths, the following potential limitations have to be considered. The study was designed as a single-dose study and did not assess any dose-response relationship. Further studies are needed to assess the full potential of AT278 U500, including studies at lower, clinically relevant doses or while continuing basal insulin, meal studies, and head-to-head studies comparing AT278 U500 with regular human insulin U500 and with currently marketed faster-acting insulin analogs. Single doses of AT278 U500 and IAsp U100 were prepared and administered using a standard U100 pediatric syringe for both study medications. As the error in dosing accuracy increases with decreasing the dose volume, all doses were prepared gravimetrically. The accuracy of the administered dose was high for both study medications (Supplementary Table 6), and the variation from the intended dose was well within the allowed graduation tolerance according to the International Organization for Standardization Standard ISO 8537:2016(E) (35) for both study medications. The difference in dosing between AT278 U500 and IAsp U100 was statistically significant (−0.002 units/kg [95% CI −0.004 to −0.001]; P = 0.0107) but is considered clinically negligible and not to compromise interpretation of the results. Furthermore, by using the U100 pediatric syringe, the maximum dose was limited to 30 units to achieve single-dose administration of 0.3 units/kg IAsp U100.

Therefore, participants with a body weight of >100 kg were excluded. This restriction in body weight and the restriction to study people with type 1 diabetes who were all male (based on regulatory recommendations for early-phase studies) (33) resulted in a study population that is not representative of those to whom a U500 prandial insulin would be prescribed. Thus, the generalization of our study results to the relevant clinical population has to be done with caution and requires further investigations (i.e., studies in insulin-resistant people with type 2 diabetes and high insulin demands as well as in women).

In conclusion, the study demonstrated that AT278 U500 offers rapid-acting characteristics in a reduced dose volume, with an accelerated absorption and onset of action when compared with IAsp U100. No significant safety findings were observed throughout the study. Our results suggest that AT278 U500 will benefit people with high insulin needs by improving blood glucose management and adding more convenience to their daily diabetes management.

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