Humalog Mix25 improves 24-hour plasma glucose profiles compared with the human insulin mixture 30/70 in patients with type 2 diabetes mellitus

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Objective. To compare the effects of Humalog Mix25 (Humalog Mix75/25 in the USA) (Mix25) and human insulin 30/70 (30/70) on the 24-hour inpatient plasma glucose (PG) profile in patients with type 2 diabetes mellitus (T2DM).

Design. A randomised, open-label, 8-week crossover study. Study insulins were injected twice daily, 5 minutes before breakfast and dinner.

Setting. Four-week outpatient (dose-adjustment) treatment phase, and 3-day inpatient (test) phase.

Patients. Twenty-five insulin-treated patients with T2DM (ages 40 - 66 years), mean (± standard error of the mean) (SEM) HbA_{1c} 7.7% ± 0.23%, and body mass index (BMI) 29.3 ± 0.83 kg/m^2.

Outcome measures. 24-hour PG profiles, PG excursions after meals, PG area under the curve (AUC), and 30-day hypoglycaemia rate.

Results. The 2-hour PG excursions following breakfast (5.5 ± 0.34 v. 7.2 ± 0.34 mmol/l, $p = 0.002$) and dinner (2.4 ± 0.27 v. 3.4 ± 0.27 mmol/l, $p = 0.018$) were smaller with Mix25 than with 30/70. PG AUC between breakfast and lunch was smaller with Mix25 than with 30/70 (77.6 ± 3.8 v. 89.5 ± 4.3 mmol/h/ml, $p = 0.001$). PG AUC between lunch and dinner, dinner and bedtime, and bedtime and breakfast did not differ between treatments. Pre-meal and nocturnal PG were comparable. The postprandial insulin requirement for lunch meals was supplied equally by the two insulin treatments. The thirty-day hypoglycaemia rate was low (Mix25 0.049 ± 0.018 v. 30/70 0.100 ± 0.018 episodes/patient/30 days, $p = 0.586$) for both treatments.

Conclusion. In patients with T2DM, Mix25 improved the 24-hour PG profile with lower postprandial PG excursions than with human insulin 30/70.

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Most patients with type 2 diabetes mellitus (T2DM) administer insulin twice daily, yet many use premixed insulin preparations (Lilly Health, data on file). Premixtures of short- or rapid-acting and intermediate-acting insulins twice daily are commonly used, offering convenience and increased dosing accuracy compared with insulins mixed from separate vials.[1] Injection of human insulin mixtures is recommended 30 - 45 minutes pre-meal to control postprandial blood glucose (BG).[2] In contrast, manufactured mixtures containing rapid-acting insulin lispro offer the convenience and accurate dosing of a premixed formulation plus immediate pre-meal or post-meal injection.

A manufactured mixture, 25% insulin lispro and 75% neutral protamine lispro (NPL) (Humalog Mix25, Humalog Mix 75/25 in the USA(Mix25)) has been approved for clinical use in most countries. The intermediate-acting insulin within these mixtures consists of a crystalline suspension of insulin lispro-protamine crystals referred to as NPL. The activity profile of NPLis similar to that of neutral protamine Hagedorn (NPH).[3] NPLis used since an exchange between soluble insulin lispro and protamine-bound human insulin occurs with prolonged storage of insulin lispro-NPH mixtures.

Compared with human insulin mixtures, Mix25 administered twice daily in T2DM patients results in improved postprandial glycaemic control, similar overall glycaemic control, less risk of nocturnal hypoglycaemia, and the convenience of dosing immediately before (or after) meals.[4-7] Nevertheless, the pharmacodynamic profile of Mix25 compared with human insulin has not been investigated over several days in a controlled clinical setting in T2DM patients.

The present pilot study compared the effects of Mix25 and human insulin 30/70 (30/70) on 24-hour plasma glucose (PG) profiles during 3 days of an inpatient test phase and frequency of hypoglycaemia throughout the study in T2DM patients.

Methods

Patient population

All patients eligible for the study had T2DM according to World Health Organisation (WHO) criteria,[8] were aged 40 - 70

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years, and had a haemoglobin A_{1C} (HbA_{1C}) < 10% (local laboratory normal reference range 4.4 - 6.4%). They had been treated with human insulin 30/70 twice daily and practised self-monitoring of BG for at least 3 months before the study. Patients were excluded if they usually injected human insulin 30 - 45 minutes before meals. With the exception of having T2DM, patients were healthy. Patients with a body mass index (BMI) > 35 kg/m^2, and those being treated with oral anithyperglycaemic agents, systemic glucocorticoids, or insulin doses > 2.0 U/kg were excluded.

Study design

This randomised, open-label, two-way crossover study consisted of a 2-week lead-in and two 4-week treatment phases (Fig. 1). Each 4-week outpatient treatment (dose-adjustment) phase preceded a 3-day inpatient (test) phase. During the inpatient phase, patients were hospitalised for up to 96 hours to determine PG profiles on 3 consecutive days. The study insulins (Eli Lilly and Company, Indianapolis, Indiana, USA) were injected 5 minutes before breakfast and dinner, omitting a lunch injection, since a twice-daily insulin regimen was investigated in this study. The ethical review board of the University of the Free State, Bloemfontein, approved the protocol, and all patients gave informed consent according to Good Clinical Practice Guidelines and the Declaration of Helsinki.

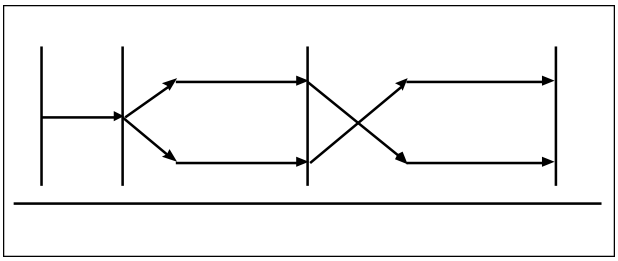

Fig. 1. Study design.

Assessments

Lead-in phase

At visit 1, a comprehensive history and physical examination was completed, blood samples were collected and study diaries were given. Patients received 30/70 before the morning and evening meals during the lead-in phase. The investigators telephoned patients at least once weekly to meet target BG for fasting and before meals (< 7.0 mmol/l) and 2 hours after breakfast and dinner (< 10.0 mmol/l). At visit 2 patients were randomised to Mix25 followed by 30/70, or 30/70 followed by Mix25.

Outpatient treatment (dose-adjustment) phase

During treatment with either insulin, patients were instructed to attempt to meet the abovementioned glycaemic goals. The investigator contacted the patients twice weekly during each treatment period to optimise insulin dose.

Between visits 2 and 3, and between visits 3 and 4, patients obtained a self-monitored BG profile twice weekly using the BG meter provided (Accutrend alpha, Boehringer Mannheim GmbH, Mannheim, Germany). Self-monitored BG profiles consisted of fasting, before lunch and dinner, and 2-hour postprandial BG after each meal. Measured BG values were used to adjust the insulin dose. A hypoglycaemic episode was defined as any time a patient experienced, or another person observed a patient experiencing a self-assessed sign/symptom associated with hypoglycaemia, or any spontaneous BG measurement < 3.0 mmol/l (54 mg/dl). Each patient recorded the BG level, associated symptoms, and treatment, and this information was collected at visits 2, 3 and 4.

Between visits 2 and 3 and between visits 3 and 4, patients were asked to reproduce the standard breakfast and dinner meal (similar to meals during the inpatient phase) several times at home to determine if an acceptable insulin dose had been identified that could be used before the standard inpatient breakfast and dinner. Although the upper limit for postprandial BG was < 10.0 mmol/l, an ideal insulin dose resulted in a postprandial BG < 8.0 mmol/l, and did not result in hypoglycaemia.

Inpatient treatment (test) phase

Patients reported to the research unit on the evening before the first day of the inpatient phase, injected either Mix25 or 30/70 and consumed a standard dinner. The following morning an indwelling cannula was established for blood sampling. The patient's fasting BG was determined between 06h30 and 07h00. If the fasting BG was > 8.0 mmol/l, a continuous intravenous regular human insulin infusion began in order to lower the BG to between 6.0 and 8.0 mmol/l within the following hour in order to have similar baselines between patients. On each test day if the target BG was not achieved before 08h00, breakfast was delayed until the target BG was reached and time of breakfast was noted as zero hour.

During the inpatient phase, an individualised diet providing 130 kJ (32 kcal) per kilogram ideal body weight was calculated (50% carbohydrate, 20% protein, and 30% fat). The carbohydrate was distributed as follows: 30% at breakfast, 30% at lunch, and 40% at dinner. *Ad libitum* consumption of non-caloric liquids (e.g. diet cola, black coffee) was allowed during the inpatient phase, but no other food was permitted.

Patients received a standard breakfast, lunch, and dinner between 07h00 and 08h00, 12h00 and 13h00, and 18h00 and 19h00, respectively, or later if more time was required to achieve the baseline target BG. Both study insulins were injected subcutaneously into the abdominal wall, 5 minutes

before breakfast and dinner. Insulin dose on day 1 was based on BG results with standard meals during the outpatient phase and remained the same on each inpatient day. However, if the BG profile determined on day 1 indicated that the dose was not effective in reaching the target BG, the dose was adjusted for day 2. In this case, insulin doses were identical on day 2 and day 3.

Two blood samples were obtained before meals and at 1-hour intervals thereafter. One sample of venous whole blood collected with sodium fluoride was used for enzymatic determination of PG and statistical analysis of the PG profiles. The other sample of venous whole blood collected with ethylenediamine tetraacetic acid (EDTA) was used for the immediate determination of BG using a BG meter. If BG was < 3.0 mmol/l and/or symptoms of hypoglycaemia occurred any time during the inpatient phase, the patient ate a standard snack. One snack unit (three Cream Cracker biscuits, Bakers Pty (Ltd), South Africa) provided 369 kJ (88 kCal). If hypoglycaemia continued, as indicated by BG measurements in 10-minute intervals until BG was > 3 mmol/l for two consecutive measurements, one more snack was eaten. Snacks were consumed as necessary to maintain BG > 3 mmol/l.

Following the collection of the final blood sample at 08h00 the morning after day 3 and before discharge from the research unit, the patient was given breakfast and the first dose of study insulin for the next treatment interval (visit 3) or the usual (prescribed) insulin (visit 4). The study was completed the morning after inpatient phase day 3 of visit 4.

Statistical methods

Following the intent-to-treat approach, data were used from all randomised patients who received at least one treatment dose. The last observation carried forward was used to impute missing data. PG and parameters computed from PG, insulin doses, and 30-day hypoglycaemia rate were analysed using the crossover method described by Koch[9] and Taulbee.[10] Analysis of variance models (ANOVAs) were used to examine the carryover and treatment effect as described in Koch[9] and Taulbee.[10] All tests were performed using a two-sided test with an alpha level of 0.05.

Glucodynamic evaluations

Parameters computed from the PG measurements included 24-hour PG profiles, and the maximum glucose concentration (C_{max}). Additional parameters based on glucose excursions from baseline were also computed. Excursions from baseline were defined as the baseline (time = 0) PG concentration subtracted from each of the measured PG concentrations. Log transformations were used to analyse the glucodynamic measurements.

Results

Patient characteristics

Of the 25 patients randomised, 21 completed the study. Four patients discontinued the study; 3 based on the investigator's decision (1 patient on Mix25 and 2 patients on 30/70), and 1 based on the patient's own decision (Mix25). There were no differences in patients' baseline characteristics (Table I). No significant unequal carryover effects were observed.

Table I. Patient baseline characteristic (mean ± SEM)

	Sequence		
	Mix25 30/70	30/70 Mix25	
Baseline variable	(*N* = 13)	(*N* = 12)	*p*-value
Gender (M/F)	10/3	7/5	0.411
Age (yrs)	54.8 ± 1.82	53.6 ± 2.15	0.667
BMI (kg/m^2)	29.2 ± 1.2	29.3 ± 1.2	0.962
HbA_{1C} (%)	7.81 ± 0.33	7.60 ± 0.33	0.645

MF = male/female; BMI = body mass index; HbA_{1C} = haemoglobin A_{1C}.

There were no differences in the mean (± standard error of the mean (SEM)) insulin doses for the two study insulins before breakfast (Mix25 31.6 ± 3.0 units v. 30/70 32.3 ± 3.4 units, $p = 0.58$) or before dinner (Mix25 26.8 ± 3.1 units v. 30/70 26.4 ± 3.2 units, $p = 0.61$) during the outpatient phase. During the inpatient phase, the insulin dose was modified from day 1 to day 2 in some patients (pre-breakfast 30/70 $N = 11$, Mix25 $N = 7$, pre-dinner 30/70 $N = 7$, Mix25 $N = 4$). Therefore, only data from days 2 and 3 were used for analysis of efficacy (PG), as the protocol required insulin doses to be the same on all inpatient test days. There were no differences in insulin doses before breakfast (Mix25 32.4 ± 3.3 units v. 30/70 33.3 ± 3.4 units, $p = 0.169$) or before dinner (Mix25 27.6 ± 3.3 units v. 30/70 27.5 ± 3.2 units, $p = 0.769$) during days 2 and 3.

Glucodynamics

The 24-hour PG profiles for the two treatments were compared (Fig. 2). Two-hour PG excursions following breakfast ($p = 0.002$) and dinner ($p = 0.018$) were significantly smaller with Mix25 than with 30/70 (Fig. 3). Fasting, pre-lunch, and pre-dinner PG levels were similar between treatments.

The PG AUC between breakfast and lunch was smaller with Mix25 than with 30/70 (Mix25 77.6 ± 3.8 mmol/h/ml v. 30/70 89.5 ± 4.3 mmol/h/ml, $p = 0.001$). The PG AUC between lunch and dinner (Mix25 131.7 ± 5.7 mmol/h/ml v. 30/70 132.6 ± 7.8 mmol/h/ml, $p = 0.789$), dinner and bedtime (Mix25 52.7 ± 3.2 mmol/h/ml v. 30/70 53.0 ± 7.8 mmol/h/ml, $p = 0.975$), and bedtime and breakfast (Mix25 117.8 ± 6.5 mmol/h/ml v. 30/70

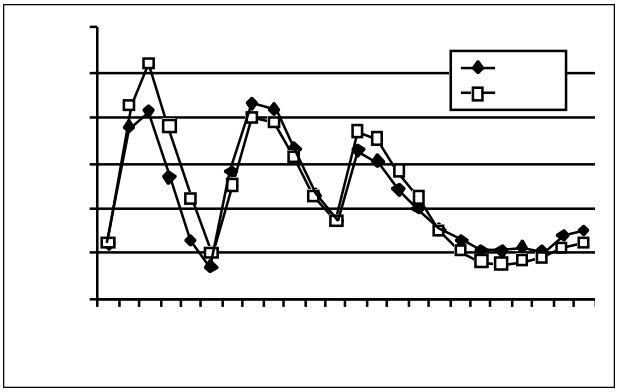

Fig. 2. 24-hour plasma glucose profiles on inpatient days 2 and 3.

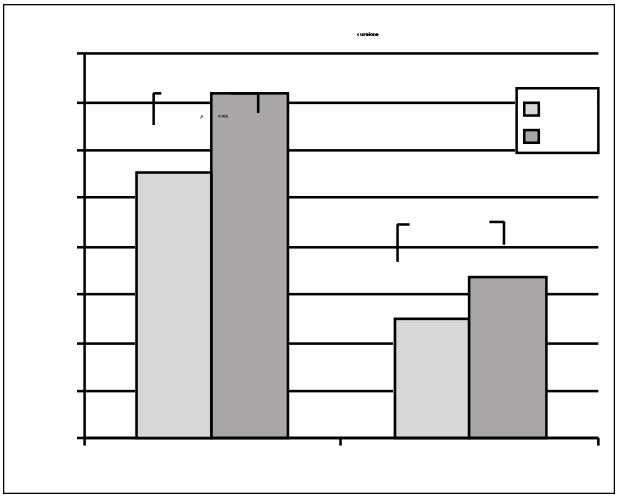

Fig. 3. 2-hour plasma glucose excursions following breakfast and dinner on inpatient days 2 and 3.

119.2 ± 9.1 mmol/h/ml, $p = 0.895$) were not different between treatments.

The C_{max} between breakfast and lunch was significantly lower with Mix25 (Mix25 13.3 ± 0.6 mmol/l v. 30/70 15.2 ± 0.7 mmol/l, $p = 0.002$) than with 30/70. The C_{max} for the remaining time intervals — lunch to dinner (Mix25 13.9 ± 0.6 mmol/l v. 30/70 13.8 ± 0.8 mmol/l, $p = 0.552$), dinner to bedtime (Mix25 12.2 ± 0.7 mmol/l v. 30/70 12.9 ± 0.8 mmol/l, $p = 0.212$), and bedtime to breakfast (Mix25 9.5 ± 0.7 mmol/l v. 30/70 10.1 ± 0.9 mmol/l, $p = 0.656$) — were not significantly different between treatments.

Hypoglycaemia

The 30-day hypoglycaemia rate was low during both the outpatient phase (Mix25 0.049 ± 0.018 episodes/patient/30 days v. 30/70 0.100 ± 0.018 episodes/patient/30 days, $p = 0.586$) and the inpatient phase (Mix25 0.241 ± 0.053 episodes/patient/30 days v. 30/70 0.222 ± 0.053 episodes/patient/30 days, $p = 0.524$) for both treatments.

Discussion

We found that the 24-hour PG profile appeared smoother with Mix25 than with 30/70 (Fig. 2). We attributed this to the PG excursions following breakfast and dinner that were smaller with Mix25 than with 30/70. Whereas the postprandial PG excursions were improved with Mix25 because of the faster onset of action of insulin lispro, PG in the late postprandial phase, before meals, and throughout the night were similar between the two study insulins. In the present study, insulin was not administered before lunch. PG after lunch was similar for Mix25 and 30/70. Therefore, the postprandial insulin requirement for lunch was supplied equally by the two insulin regimens. The rates of hypoglycaemia during the inpatient and outpatient phases were low and did not differ between treatments.

The present findings are in agreement with others.[4,6,7] In a previous study of T2DM patients, Mix25 provided better postprandial BG control than either 30/70 or NPH, following a standard test meal;[6] specifically, Mix25 significantly lowered the 4-hour glucose AUC and the maximum glucose excursion. Malone *et al.*[7] confirmed the findings of that study, reporting smaller BG excursions with Mix25 following a standard test meal in T2DM patients. Roach *et al*.[4] also reported that twice-daily administration of Mix25 in T2DM patients resulted in improved postprandial glycaemia control and similar overall glycaemic control, while providing the convenience of administering insulin immediately before meals compared with 30/70. The present study agrees with these previous findings and provides further evidence supporting the use of Mix25 in T2DM patients.

Increasing evidence supports the significance of postprandial BG and the importance of its control in preventing some of the long-term complications associated with diabetes.[11-13] Therefore, treatment regimens that provide superior postprandial control become increasingly important. Indeed, the Diabetes Control and Complications Trial Research Group (DCCT) investigators speculated that 'mean HbA_{1c} is not the most complete expression of the degree of hyperglycaemia. Other features of diabetic glucose control, which are not reflected by HbA_{1c}, may add to or modify the risk of complications. For example, the risk of complications may be more highly dependent on the extent of postprandial glycemic excursions.'[14]

A possible limitation of the present study was that both study insulins were injected 5 minutes before breakfast and dinner; however, evidence suggests that injecting insulin close to the time of eating is the practice of the majority of patients.[15] It is recommended that 30/70 be injected 30 minutes before meals. If this recommended time of injection was used for

30/70 in the present study, the difference between the effects of the two study insulins might have been less pronounced. However, the timing of injection used in the present study was considered to be a more realistic approach based on clinical experience. It may be of interest to re-examine the research question including a separate arm of the study with a 30-minute pre-meal injection of 30/70.

Conclusion

Mix25 provided a smoother 24-hour PG profile with smaller PG excursions following breakfast and dinner compared with 30/70. The rate of hypoglycaemia throughout the study was low and not significantly different for the two study insulins. Therefore, Mix25 is a valuable treatment option for patients with T2DM.

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