28-week, randomized, multicenter, open-label, parallel-group phase III trial to investigate the efficacy and safety of biphasic insulin aspart 70 thrice-daily injections *vs* twice-daily injections of biphasic insulin aspart 30 in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: An insulin analogue formulation with a 7:3 ratio of rapid-acting and intermediate-acting fractions, biphasic insulin aspart 70 (BIAsp70) was developed to supplement basal insulin between meals and mimic the physiological pattern of postprandial insulin secretion.

Materials and Methods: We carried out a randomized, open-label study to compare the efficacy and safety profiles of BIAsp70 and an insulin analogue formulation with a 3:7 ratio of rapid-acting and intermediate-acting fractions (BIAsp30) in type 2 diabetes mellitus patients. Patients were randomized and received either thrice-daily BIAsp70 (n = 145) or twice-daily BIAsp30 (n = 144) for 28 weeks. The primary end-point was glycated hemoglobin (HbA_{1c}) after 16 weeks of treatment.

Results: Non-inferiority of BIAsp70 *vs* BIAsp30 was confirmed and superiority was established with a between-group difference (BIAsp70–BIAsp30) in HbA_{1c} after 16 weeks of treatment of −0.35% (95% CI: −0.51 to −0.19; $P < 0.0001$ for superiority). The mean postprandial glucose increment (19.96 *vs* 54.35 mg/dL; $P < 0.0001$) and M-value (12.99 *vs* 17.94; $P < 0.0001$) at 16 weeks were smaller in the BIAsp70 group than in the BIAsp30 group, and were maintained at 28 weeks. Pre-breakfast glucose (157.9 *vs* 140.7 mg/dL), total insulin dose (46.8 *vs* 38.1 U/day) and weight gain (+1.94 *vs* 1.23 kg) at week 28 were greater in the BIAsp70 group. Incidence of nocturnal hypoglycemia was significantly lower with BIAsp70 *vs* BIAsp30 (1.23 *vs* 3.21 events/subject year; $P = 0.0002$) at week 28.

Conclusions: Thrice-daily BIAsp70 was superior to twice-daily BIAsp30 in terms of HbA_{1c} change, with less variation in daytime plasma glucose profiles. BIAsp70 was well tolerated, with a lower incidence of nocturnal hypoglycemia *vs* BIAsp30. This trial was registered with ClinicalTrial.gov (no. NCT00318786). (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00015.x, 2010)

KEY WORDS: Biphasic insulin aspart 70, Postprandial hyperglycemia, Type 2 diabetes mellitus

INTRODUCTION

A number of landmark clinical trials have conclusively verified that rigorous glycemic control with intensive insulin therapy significantly reduces the onset and rate of progression of complications[1–3], and suggest that approximating a physiological insulin secretion pattern through supplementation with exogenous insulin inhibits the progression of complications. It is also known that excessive postprandial blood glucose concentration and impaired glucose tolerance, as estimated by the glucose concentration 2 h after an oral glucose tolerance test, are significant risk factors for the onset and progression of cardiovascular disease[4,5], and that addressing postprandial glucose is an important therapeutic target to reduce the risk or progression of cardiovascular disease[6–8]. Therefore, besides lowering glycated hemoglobin (HbA_{1c}), inhibiting fluctuations of blood glucose levels are also important goals for preventing the development and progression of macrovascular and microvascular complications.

One of the most widely-used insulin therapies for type 2 diabetes in Japan is twice-daily treatment with mixed insulin formulations containing both rapid-acting and intermediate-acting fractions in the ratio 3:7; this strategy offers effective glycemic control for many patients, with a small number of daily injections[9,10]. In patients in whom this approach

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inadequately controls glycemia during the day, and in those whose pathological state is more advanced, a basal-bolus therapeutic regimen is currently favored. However, this approach requires 4–5 injections/day, which, in addition to the perceived complexity of the regimen, might have negative effects on quality of life, treatment satisfaction and compliance relative to twice-daily mixed insulin[11].This situation has led to the innovation of thrice-daily (preprandial) administration of rapid-acting insulin analogues[12], although the insulin action might drop away and blood glucose levels rise between successive injections.

For example, in one clinical study of Japanese patients with type 1 diabetes using a basal-bolus regimen with insulin aspart as the rapid-acting insulin analogue, the average ratio of bolus and basal insulin was approximately 7:3[13]. Therefore, we hypothesized that a ratio of 7:3 would be more appropriate than a ratio of 3:7 in Japanese patients. However, because insulin demand, dietary habits and lifestyle factors vary among individual patients, it was hypothesized that administration of biphasic insulin aspart 70 (BIAsp70) alone or in combination with a biphasic insulin analogue containing a rapid-acting fraction and an intermediate-acting fraction in the ratio 3:7 (BIAsp30) thrice daily might be a suitable alternative regimen that would allow physicians to cater to diverse requirements for insulin therapy among diabetes patients.

Therefore, the present randomized, multicenter, open-label, parallel-group trial was carried out to compare the efficacy and safety of thrice-daily BIAsp70 with those of BIAsp30 given twice daily.

MATERIALS AND METHODS

Subjects

The subjects of the present trial were patients with type 2 diabetes mellitus having HbA_{1c} levels between 7.5% and 10.0%, and who were previously receiving once- or twice-daily injections of an intermediate-acting human insulin preparation, long-acting insulin analogue or a mixed human insulin preparation. Before their participation in the trial, written informed consent was obtained from all patients. The study protocol was reviewed and approved by institutional review boards at each of the participating trial sites (Appendix S1), and the trial was carried out in accordance with the Declaration of Helsinki and with Japanese Good Clinical Practice and other applicable regulations.

Investigational Products

BIAsp70 and BIAsp30 were given in a 3-mL cartridge formulation containing 100 U of active ingredient per mL. The investigational products were manufactured by Novo Nordisk A/S (Bagsvaerd, Denmark) and supplied by Novo Nordisk Pharma Ltd (Tokyo, Japan).

Methodology

The primary objective was to show non-inferiority of thrice-daily (before each meal) BIAsp70 *vs* twice-daily (before breakfast and dinner) BIAsp30 for glycemic control as shown by HbA_{1c} after 16 weeks of treatment. HbA_{1c} was measured by high-performance liquid chromatography, and all HbA_{1c} data are shown in Japan Diabetes Society (JDS) values.

Secondary efficacy end-points were HbA_{1c} after 28 weeks of treatment, proportion of subjects achieving HbA_{1c} <6.5% at 16 and 28 weeks, and plasma glucose (PG) profile at seven daily time-points (immediately before and 120 min after each meal and before bedtime) at 16 and 28 weeks. PG profile included mean PG, mean postprandial PG (PPG) increment, and *M*-value[14] (an index of PG fluctuation that shows the degree of deviation from the ideal blood glucose value [120 mg/dL] and shows the degree of diurnal variation).

Safety evaluation variables were incidence of hypoglycemia and adverse events, and bodyweight. The insulin dose and presence of insulin antibodies were also investigated. Episodes of hypoglycemia were to be recorded by the subject in a diary, along with any symptoms, PG value at the time, treatments used and whether the subject was able to treat him/herself. Episodes were classified according to the following scheme:

1 Minor hypoglycemia: hypoglycemia with signs or symptoms that could be personally managed by the subject (blood glucose ≤55 mg/dL).
2 Symptoms-only hypoglycemia: hypoglycemia with signs or symptoms that could be personally managed by the subject (blood glucose not measured or ≥56 mg/dL).
3 Biochemical hypoglycemia: hypoglycemia not accompanied by signs or symptoms (blood glucose ≤55 mg/dL).
4 Major hypoglycemia: hypoglycemia necessitating treatment by a third party owing to serious central nerve disorders induced by hypoglycemia.

For both groups, the daily total insulin dose at the start of treatment was taken as that prescribed in the subject's prior therapeutic regimen. In cases where the subject's prior treatment was once-daily long-acting insulin analogue or NPH human insulin, the starting dose was determined by the investigator and sub-investigator.

After starting the study treatment, the insulin dose could be adjusted by the investigator at each visit/telephone contact (every 1–2 weeks) to maintain glycemic control throughout the treatment period based on information recorded in the patient's diary (BG measurements, hypoglycemia and seven-point PG profiles carried out on at least two days the week before each visit/telephone contact). Insulin doses in both groups were titrated using a recommended (but not enforced) algorithm to achieve a fasting PG <130 mg/dL and 2-h PPG <180 mg/dL (Supporting Information Table S1). The investigational products were injected immediately before meals in both groups. In the BIAsp70 group, subjects who failed to achieve the target pre-breakfast PG level of <130 mg/dL at 16 weeks could have their pre-dinner formulation switched to BIAsp30.

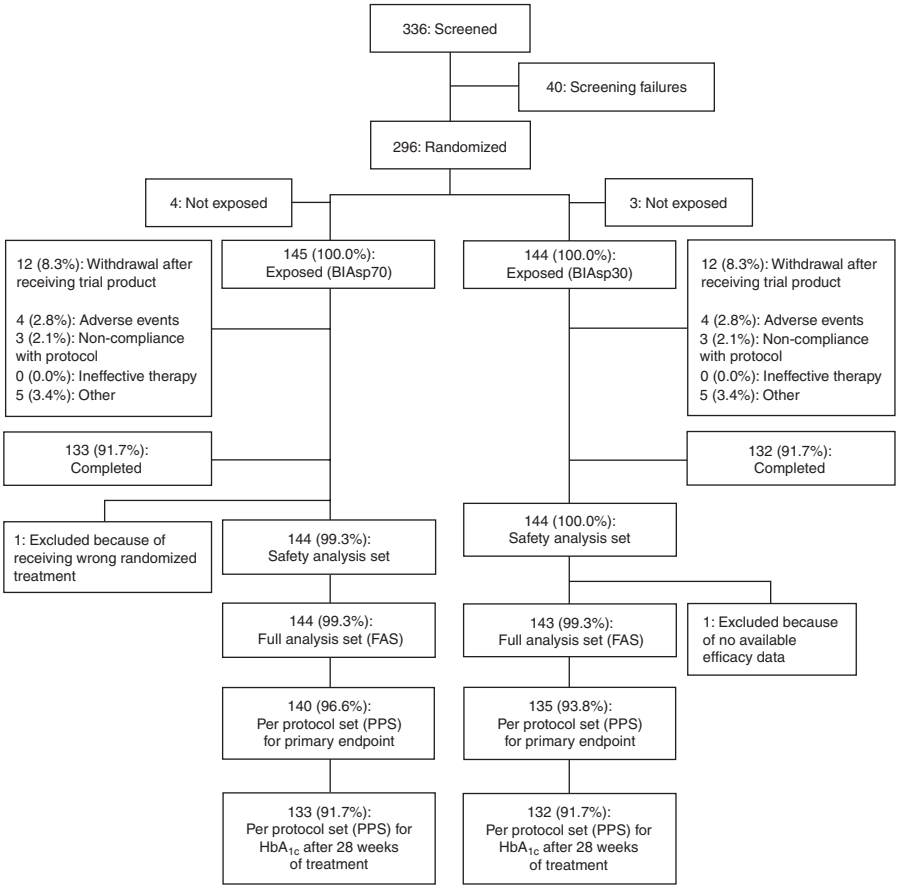

Figure 1 | Disposition of subjects in the trial.

Statistical Analysis

Efficacy was assessed on the basis of the full analysis set (FAS) data. The primary end-points, HbA_{1c} level after 16 weeks of treatment and HbA_{1c} level after 28 weeks, were also assessed using the per-protocol set (PPS) data. Safety end-points were analyzed for all subjects who received the investigational product at least once. Missing data were imputed using the last observation carried forward (LOCF) approach for end-points at 16 and 28 weeks.

The primary end-point was assessed by an analysis of variance (ANOVA) with baseline HbA_{1c} as a covariate and treatment group as a fixed effect. Based on this model, the two-sided 95% confidence interval (CI) for treatment differences (BIAsp70–BIAsp30) was calculated; if the upper bound of the 95% CI of the FAS and PPS data was <0.4% of the non-inferiority margin, BIAsp70 was regarded as non-inferior to BIAsp30. Furthermore, if non-inferiority was shown, between-group superiority was to be evaluated using the above CI. HbA_{1c} level after 28 weeks of treatment was analyzed and evaluated using the same procedure as the primary end-point.

The end-points derived from the seven-point daily PG profile (mean PG, mean PPG increment and *M*-value) at 16 and 28 weeks were analyzed using ANOVA models with the corresponding baseline value as a covariate and treatment group as a fixed effect. *M*-value was analyzed after log-transformation. Statistical tests were carried out only for the end-points at 16 weeks.

The proportions of subjects in each group achieving HbA_{1c} <6.5% after 16 weeks of treatment were compared by χ^2-test. For the proportion of subjects achieving HbA_{1c} <6.5% after 28 weeks of treatment, the CI of the differences in proportion based on a normal approximation of a binomial distribution were shown.

For the daily insulin dose, the 95% CI of the between-group differences in the changes from baseline to 16 and 28 weeks were calculated.

The incidence of total and nocturnal hypoglycemia was analyzed using a generalized linear model based on a Poisson distribution, and the 95% CI of relative risk was determined. Change in bodyweight was compared between groups using two-sample *t*-test. Changes in the levels of insulin antibodies were compared between groups using Wilcoxon's test.

RESULTS

Subjects

The disposition of subjects in the present trial is shown in Figure 1. In the present study, 336 subjects were screened. Of these, 296 subjects were randomized and 289 subjects received the investigational product. Twelve subjects (8.3%) in each

Table 1 | Demographics and other baseline characteristics

Item	BIAsp70 (n = 144)	BIAsp30 (n = 144)
Sex (male/female), *n* (%)	89 (61.8)/55 (38.2)	81 (56.3)/63 (43.8)
Age (years)	62.4 ± 9.3	62.7 ± 9.7
Bodyweight (kg)	63.57 ± 10.05	60.43 ± 9.66
BMI (kg/m^2)	24.27 ± 2.65	23.63 ± 2.84
Duration of diabetes (years)	15.98 ± 8.58	15.48 ± 8.09
No. prior daily insulin injections, *n* (%)		
Once daily	11 (7.6)	10 (6.9)
Twice daily	133 (92.4)	134 (93.1)
Total daily insulin dose, U/day	29.0 ± 12.5	26.5 ± 10.5
HbA_{1c} (%)	8.50 ± 0.72	8.47 ± 0.76

Means ± SD unless otherwise indicated. BIAsp, biphasic insulin aspart; BMI, body mass index; HbA_{1c}, glycated hemoglobin.

group were withdrawn after receiving the investigational product.

One subject from the BIAsp70 group who received the incorrect randomized treatment was excluded from both the safety analysis set and the FAS. In addition, one subject from the BIAsp30 group who had no available efficacy data after treatment was excluded from the FAS.

There were no major differences between the two groups with respect to demographics and other baseline characteristics (Table 1).

Efficacy

HbA_{1c}. In both groups, mean HbA_{1c} gradually decreased from baseline to 20 weeks after starting treatment, and thereafter remained approximately unchanged (Figure 2). In the BIAsp70 group, mean HbA_{1c} (mean ± SD) decreased from 8.50 ± 0.72% at baseline to 7.22 ± 0.71% at 16 weeks and 7.18 ± 0.71% at the end of treatment; whereas in the BIAsp30 group, the levels were 8.47 ± 0.77%, 7.56 ± 0.75% and 7.48 ± 0.78% at the same time-points, respectively.

The point estimate of the between-group difference (BIAsp70 − BIAsp30) in HbA_{1c} at 16 weeks was −0.35% (95% CI: −0.51 to −0.19, $P < 0.0001$; FAS). Thus, it was shown that thrice-daily BIAsp70 is not only non-inferior, but also superior to twice-daily BIAsp30. Essentially, the same result was obtained in the subsequent analysis of the PPS data.

Likewise, the point estimate between-group difference of HbA_{1c} at 28 weeks adjusted for the baseline value was −0.30% (95% CI: −0.47 to −0.14, $P = 0.0004$; FAS), showing non-inferiority of thrice-daily BIAsp70 relative to twice-daily BIAsp30, as well as superiority.

The proportion of subjects achieving the HbA_{1c} target of <6.5% at 16 weeks was 20/144 subjects (13.9%) in the BIAsp70 group and 7/143 subjects (4.9%) in the BIAsp30 group (Figure S1). A between-group comparison showed a statistically significant difference ($P = 0.0091$).

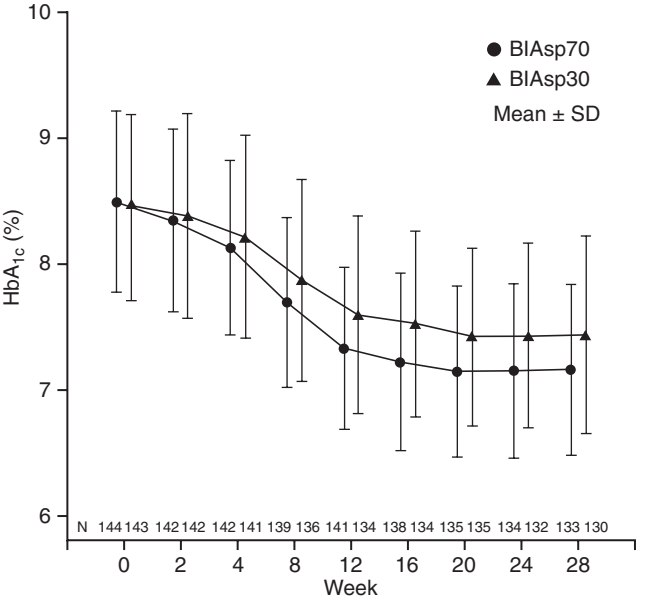

Figure 2 | Mean profiles of HbA_{1c} in the two groups throughout the study.

The proportions of subjects achieving a HbA_{1c} <6.5% at 28 weeks were 23/144 (16.0%) in the BIAsp70 group and 17/143 (11.9%) in the BIAsp30 group.

Investigation of Subgroups Based on a Change in Pre-Dinner Injection

Among the 144 subjects in the BIAsp70 group, the pre-dinner formulation was switched to BIAsp30 after 16 weeks of treatment in 96 subjects (66.7%). Subjects who changed their pre-dinner formulation to BIAsp30 showed a slightly higher HbA_{1c} at baseline in comparison with those who did not change their formulation (the mean value for subjects who changed their pre-dinner formulation and those who did not were 8.61 ± 0.69% and 8.27 ± 0.73% respectively). Mean HbA_{1c} at 16 weeks was 7.25 ± 0.71%, compared with 7.16 ± 0.70% in the subgroup that did not switch.

In both subgroups, HbA_{1c} decreased until 20 weeks and thereafter remained unchanged. Mean HbA_{1c} at 28 weeks was 7.22 ± 0.70% in subjects who switched their pre-dinner formulation to BIAsp30 and 7.11 ± 0.73% in those who did not switch.

The proportion of subjects who achieved a HbA_{1c} <6.5% at 28 weeks was 13/96 (13.5%) in the subgroup that switched their pre-dinner formulation to BIAsp30 and 10/48 (20.8%) in the subgroup that did not switch.

Seven-Point Daily Plasma Glucose Profiles After 16 Weeks

Compared with the BIAsp30 group, post-lunch to post-dinner PG excursions were smaller in the BIAsp70 group and, in particular, the difference in the 120-min post-lunch PG values was substantial. In contrast, the pre-breakfast PG level in the BIAsp70 group was higher than that in the BIAsp30 group,

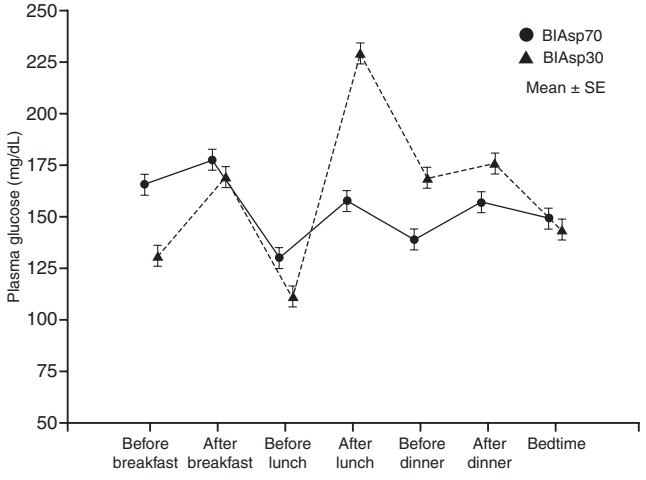

Figure 3 | Plasma glucose profiles in the two groups at 16 weeks.

although the post-breakfast PG increment was lower than that in the BIAsp30 group (Figure 3).

The mean PG level in the BIAsp70 group was significantly lower by −8.21 mg/dL (95% CI: −16.06 to −0.37; $P = 0.0402$) than that in the BIAsp30 group. Mean PPG increments at baseline and at 16 weeks were 87.24 ± 50.28 and 19.88 ± 43.02 mg/dL, respectively, in the BIAsp70 group, and 87.92 ± 50.26 and 54.43 ± 49.57 mg/dL, respectively, in the BIAsp30 group, as determined from seven-point PG profiles (Figure 3, Figure S2). The between-group difference in mean PPG increment at 16 weeks adjusted for baseline level was −34.39 mg/dL (95% CI: −45.11 to −23.67; $P < 0.0001$).

In the BIAsp70 group, mean PG level at 120 min after each meal was within the range for 'good' glycemic control (140–180 mg/dL) according to the indices proposed by JDS[15].

The estimated treatment ratio (BIAsp70/BIAsp30) of *M*-values at 16 weeks adjusted for baseline level was 0.72 (95% CI: 0.62 to 0.84, $P < 0.0001$), suggesting that a significantly smaller variation of diurnal PG levels is produced by thrice-daily BIAsp70.

Seven-Point Daily Plasma Glucose Profiles After 28 Weeks

The seven-point plasma glucose profiles at 28 weeks are shown in Figure S2. The point estimate of the between-group difference in mean PG levels at 28 weeks, adjusted for baseline levels, was −5.87 mg/dL (95% CI: −14.16 to 2.41).

The mean PPG increments at baseline and at 28 weeks were 86.90 ± 49.75 and 22.80 ± 41.28 mg/dL, respectively, in the BIAsp70 group and 87.37 ± 50.48 and 47.47 ± 44.37 mg/dL, respectively, in the BIAsp30 group. Mean PPG increment at 28 weeks was lower in the BIAsp70 group than in the BIAsp30 group (treatment difference, −24.55 mg/dL [95% CI: −34.38 to −14.73]). Thus, the same result as after 16 weeks was obtained.

Mean PG level at 120 min after each meal in the BIAsp70 group was within the range for 'good' glycemic control according to JDS[15].

The point estimate of the estimated treatment ratio of *M*-values at 28 weeks was 0.72 (95% CI: 0.60 to 0.85), suggesting that thrice-daily BIAsp70 or BIAsp70 taken before breakfast and lunch plus BIAsp30 before dinner resulted in lower diurnal variations in PG levels than the BIAsp30 twice-daily regimen.

Insulin Dose

The daily total insulin doses at baseline and after 15 and 26 weeks of treatment were 29.0 ± 12.5, 45.8 ± 18.5 and 46.8 ± 19.7 U/day, respectively, in the BIAsp70 group and 26.5 ± 10.5, 37.8 ± 16.7 and 38.1 ± 17.9 U/day, respectively, in the BIAsp30 group; showing that the mean daily total insulin dose increased primarily in the 16-week period from baseline in both groups. Furthermore, the insulin dose in the BIAsp70 group was greater than that in the BIAsp30 group during this period.

Safety

Over the 28-week treatment period, a total of 369 adverse events were reported in 120 of the 144 subjects (83.3%) in the BIAsp70 group and 342 events were reported in 119 of the 144 subjects (82.6%) in the BIAsp30 group. Adverse events for which a causal relationship with the investigational product could not be ruled out consisted of 27 events in 24 subjects (16.7%) and 27 events in 20 subjects (13.9%) in the BIAsp70 and BIAsp30 groups, respectively. Among these adverse events, the most frequently reported was diabetic retinopathy with 10 events in 10 subjects (6.9%) from the BIAsp70 group and six events in five subjects (3.5%) from the BIAsp30 group (Table 2; Table S2).

In the BIAsp70 group 12 serious adverse events were reported in 10 subjects (6.9%), and there were 12 serious adverse events in nine subjects (6.3%) from the BIAsp30 group. Excluding hypoglycemia in the BIAsp30 group (three events in two subjects), there were no serious adverse events occurring in ≥2 subjects in either group. One death occurred in the BIAsp70 group; this was as a result of complications related to the progression of diabetes and was considered unrelated to the investigational product.

Four adverse events that led to withdrawal from the trial occurred in four patients (2.8%) from each group, and consisted of gastric cancer, depression, lymphoma and cardiac death (one event of each) in the BIAsp70 group; and cerebral infarction, skin exfoliation, malignant lung neoplasm and cardiac failure (one event of each) in the BIAsp30 group.

Hypoglycemia

Over the 28-week treatment period, 1425 occurrences of hypoglycemia (18.84 events/subject years) were reported in 130 of the 144 subjects (90.3%) in the BIAsp70 group and 1594 occurrences (21.29 events/subject years) were reported in 127 of the 144 subjects (88.2%) in the BIAsp30 group. One occurrence of major hypoglycemia was noted in one subject (0.7%) in the BIAsp70 group, and four occurrences in three subjects (2.1%) in the BIAsp30 group. There was no difference between

Table 2 | Frequency distribution of adverse events with a possible or probable relationship with the trial product over the 28-week treatment period

Adverse events (preferred term)	BIAsp70 (n = 144)			BIAsp30 (n = 144)		
	n	%	*E*	*n*	%	*E*
Total	24	16.7	27	20	13.9	27
Eye disorders	12	8.3	12	7	4.9	8
Diabetic retinopathy	10	6.9	10	5	3.5	6
Cataract	2	1.4	2	0	0.0	0
Maculopathy	0	0.0	0	1	0.7	1
Retinal hemorrhage	0	0.0	0	1	0.7	1
Investigations	9	6.3	9	3	2.1	4
Weight increased	3	2.1	3	0	0.0	0
γ-GTP increased	1	0.7	1	0	0.0	0
Blood amylase increased	1	0.7	1	0	0.0	0
Blood potassium increased	1	0.7	1	0	0.0	0
Blood cholesterol increased	1	0.7	1	0	0.0	0
LDH increased	0	0.0	0	1	0.7	1
ECG						
ST-T segment abnormal	1	0.7	1	0	0.0	0
T wave inversion	1	0.7	1	0	0.0	0
Signs of ventricular hypertrophy	0	0.0	0	1	0.7	1
T wave amplitude decreased	0	0.0	0	1	0.7	1
Q wave abnormal	0	0.0	0	1	0.7	1
General disorders	2	1.4	2	3	2.1	3
Injection site urticaria	1	0.7	1	0	0.0	0
Edema peripheral	1	0.7	1	0	0.0	0
Feeling abnormal	0	0.0	0	1	0.7	1
Chest pain	0	0.0	0	1	0.7	1
Injection site induration	0	0.0	0	1	0.7	1
Metabolism/nutrition disorders	1	0.7	1	3	2.1	4
Hypoglycemia	1	0.7	1	2	1.4	3
Hyperuricemia	0	0.0	0	1	0.7	1
Vascular disorders	1	0.7	1	0	0.0	0
Hypertension	1	0.7	1	0	0.0	0
Ear/labyrinth disorders	1	0.7	1	0	0.0	0
Vertigo	1	0.7	1	0	0.0	0
Neoplasms	1	0.7	1	0	0.0	0
Gastric cancer	1	0.7	1	0	0.0	0
Nervous system disorders	0	0.0	0	2	1.4	2
Hypoglycemic coma	0	0.0	0	1	0.7	1
Diabetic neuropathy	0	0.0	0	1	0.7	1
Cardiac disorders	0	0.0	0	1	0.7	2
Atrial fibrillation	0	0.0	0	1	0.7	1
AV block first degree	0	0.0	0	1	0.7	1
Skin disorders	0	0.0	0	1	0.7	1
Hemorrhage subcutaneous	0	0.0	0	1	0.7	1
Gastrointestinal disorders	0	0.0	0	1	0.7	1
Gastric mucosal lesion	0	0.0	0	1	0.7	1
Hepatobiliary disorders	0	0.0	0	1	0.7	1
Liver function abnormal	0	0.0	0	1	0.7	1

E, number of episodes; ECG, electrocardiogram; γ-GTP, gamma-glutamyl transpeptidase; LDH, lactic dehydrogenase; *n*, number of subjects reported with adverse events; %, proportion of subjects with adverse events.

the groups with respect to the numbers of hypoglycemia events.

Over the entire treatment period, 93 events of nocturnal hypoglycemia (1.23 events/subject year) were reported in 40 of the 144 subjects (27.8%) in the BIAsp70 group, and 240 events (3.21 events/subject year) were reported in 68 of the 144 subjects (47.2%) in the BIAsp30 group. The incidence of nocturnal hypoglycemia was significantly lower in the BIAsp70 group than in the BIAsp30 group (P = 0.0002). One occurrence of major nocturnal hypoglycemia was noted in one subject (0.7%) in the BIAsp70 group and two occurrences in two subjects (1.4%) in the BIAsp30 group.

Bodyweight

Changes in bodyweight from baseline after 16 and 28 weeks were 1.44 and 1.94 kg, respectively, in the BIAsp70 group, and 0.77 and 1.23 kg, respectively, in the BIAsp30 group. A between-group comparison of the changes in bodyweight after 16 and 28 weeks showed that at each time-point the increase in weight in the BIAsp70 group was significantly greater than that in the BIAsp30 group by 0.68 kg at week 16 and 0.70 kg at week 28 (P = 0. 0110).

Antibodies

No remarkable changes of insulin aspart-specific antibodies were observed in either group during the treatment period. Insulin aspart-human insulin cross-reacting antibodies increased in both groups from baseline to 16 weeks. From 16 weeks onwards, the levels of antibodies decreased slightly in the BIAsp70 group, but increased slightly in the BIAsp30 group. A between-group comparison showed that the change in antibody titers was significantly smaller in the BIAsp70 group than in BIAsp30 group at each time-point. No obvious association between cross-reactive antibodies and HbA_{1c} or insulin doses was noted in either group at 16 and 28 weeks.

DISCUSSION

For the primary end-point, reduction of HbA_{1c} after 16 weeks of treatment, thrice-daily administration of BIAsp70 monotherapy yielded non-inferior or greater benefits compared with twice-daily BIAsp30. The proportion of subjects achieving a HbA_{1c} level <6.5% after 16 weeks of treatment, thus corresponding to 'excellent' or 'good' in the indices for glycemic control proposed by the JDS[9], was significantly higher in the BIAsp70 group than in the BIAsp30 group. From the results for each glucose-related evaluation variable at 16 weeks, the inhibitory effect on postprandial glucose increment was greater with thrice-daily BIAsp70 than with twice-daily BIAsp30, showing that PG levels after each meal were well controlled.

After 28 weeks, in an investigation that included some subjects who received combination BIAsp70 with the pre-dinner formulation switched to BIAsp30 at 16 weeks, the effect on HbA_{1c} in the BIAsp70 group was also non-inferior to or greater than that in the BIAsp30 group. The proportions of subjects

achieving a HbA_{1c} <6.5% after 28 weeks were similar in both groups. Thrice-daily BIAsp70 and twice-daily BIAsp30 significantly reduced PPG excursions and provided good control of glucose levels during the day, and the magnitude of reduction was greater with BIAsp70.

The incidence of hypoglycemia did not differ substantially between the two groups, but the risk of nocturnal hypoglycemia in the BIAsp70 group was lower than that in the BIAsp30 group. Furthermore, there were few occurrences of major hypoglycemia in both groups.

The proportions of subjects experiencing adverse events, including those related to taking the study drugs, were similar in the BIAsp70 and BIAsp30 groups. No unexpected delayed-onset events were reported. Among the adverse events for which a causal relationship with the investigational product could not be ruled out, the most commonly reported event in both groups was diabetic retinopathy. However, no severe diabetic retinopathy was reported and several of the cases showed aggravation of pre-existing diabetic retinopathy, and it has been shown that long-term improvement of glycemic control inhibits the onset and progression of retinopathy[1,16]. Furthermore, as shown in Supporting Table S2, some of the subjects had pre-existing diabetic retinopathy before commencing the study, probably related to long-term suboptimal glycemic control of the study population at baseline. Therefore, the higher incidence of diabetic retinopathy in the BIAsp70 bolus therapy group in this trial does not represent a clinically meaningful risk.

The insulin dose was adjusted aggressively at the beginning of the treatment period, and by 16 weeks it was considered that the maintenance dose had been achieved in both groups. Throughout the treatment period, the increase in insulin dose in the BIAsp70 group was greater than that in the BIAsp30 group.

A previous clinical pharmacology study carried out in type 2 diabetes patients in Japan[17] showed that thrice-daily BIAsp70 yielded a pharmacokinetic profile that was closer to the physiological insulin profile in healthy individuals as compared with twice-daily BIAsp30. Good PPG control can therefore be obtained by administering BIAsp70 immediately before meals.

Not only reducing HbA_{1c}, but also minimizing variations in PG levels through correction of postprandial hyperglycemia is important for inhibiting the onset or progression of large-vessel disease. Because BIAsp70 was shown to more significantly reduce the PPG increment than BIAsp30, this formulation might offer clinically important effects.

At present, there are several options for insulin therapy, ranging from a frequent injection regimen, such as basal-bolus therapy, to one with few injections, such as once or twice daily. Additionally, switching from biphasic human insulin, BIAsp30 has been shown to be clinically useful in insulin induction[9,13].

The results of the present study must be considered after taking into account the following limitations. First, the titration algorithm and option to change pre-dinner dose was not strictly enforced, as done in some studies. Thus, for some subjects, the dose titration after 16 weeks might have been sub-optimal. Second, this trial was not designed to evaluate the effect of switching pre-dinner formulation to BIAsp30 in the BIAsp 70 group, because the switching was carried out based on the subject's glycemic control at 16 weeks.

For patients in whom adequate glycemic control is not obtained with continuing twice-daily administration of an intermediate-acting mixed insulin formulation, basal-bolus therapy should be considered. However, some patients find it difficult to take 4–5 injections/day. Thrice-daily BIAsp70 might be a useful new treatment option for such type 2 diabetic patients before basal-bolus therapy, offering the benefit of fewer injections than basal-bolus therapy.

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SUPPORTING INFORMATION

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

Appendix S1 | List of participating institutions.
Figure S1 | Proportion of patients with HbA_{1c} <6.5% at weeks 16 and 28.
Figure S2 | Seven-point plasma glucose profiles at weeks 28.
Table S1 | Suggested algorithm for adjustment of the insulin dose
Table S2 | Assessment of diabetic retinopathy based on fundoscopy or fundus photos at baseline and at weeks 16 and 28

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