

Fixed ratio dosing of pramlintide with regular insulin before a standard meal in patients with type 1 diabetes

Amylin is co-secreted with insulin and is therefore lacking in patients with type 1 diabetes. Replacement with fixed ratio co-administration of insulin and the amylin analogue pramlintide may be superior to separate dosing. This concept was evaluated in a ratio-finding study. Patients with type 1 diabetes were enrolled in a randomized, single-masked, standard breakfast crossover study using regular human insulin injected simultaneously with pramlintide 6, 9 or 12 mcg/unit insulin or placebo. Insulin dosage was reduced by 30% from patients' usual estimates. Plasma glucose, glucagon and pramlintide and adverse events were assessed. All ratios reduced 0–3-h glucose and glucagon increments by >50%. No hypoglycaemia occurred. Adverse events were infrequent and generally mild. All pramlintide/insulin ratios markedly and safely reduced glycaemic excursions and suppressed glucagon secretion in the immediate postprandial state. Further study using one of these ratios to explore the efficacy and safety of longer-term meal-time and basal hormone replacement is warranted.

Keywords: amylin, pramlintide, type 1 diabetes

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Introduction

Amylin is co-secreted with insulin by the pancreatic β cell [1]. Its centrally mediated metabolic effects include modulation of gastric emptying, suppression of inappropriate glucagon secretion and decreased food intake [2,3]. Pramlintide, an injectable amylin analogue, is approved by the US Food and Drug Administration to supplement meal-time insulin therapy in type 1 and 2 diabetes [4]. Its limitations include the need for separate pramlintide injections in addition to daily insulin injections, and nausea after initiation of treatment. While meal-time insulin dosing is adjusted for each meal, pramlintide is usually given before meals at a fixed dosage. Hypoglycaemia can occur when doses of rapid-acting insulin and pramlintide are not well matched to a meal [5,6]. Fixed ratio co-administration of pramlintide with insulin, mimicking normal secretion and including basal as well as meal-time delivery, may prove simpler and better tolerated. Regular human insulin has shown a better postprandial glucose profile than rapid-acting analogue insulin when used with pramlintide [7]. In the present paper, we report the results of an initial study exploring this fixed ratio concept.

Methods

The study compared three pramlintide/insulin ratios with placebo/insulin, taken before a standard breakfast meal

by patients with type 1 diabetes not optimally controlled using basal-bolus therapy by insulin pump or multiple daily injections of insulin analogues. Institutional review board approval and informed consent from patients were obtained.

Each patient completed four clinic visits over a 4-week period, with a four-way crossover design. Exclusion criteria included severe hypoglycaemia in the preceding 6 months, hypoglycaemia unawareness, gastroparesis or requirement for breakfast-time insulin doses exceeding 10 U. Breakfast meals (600 kcal) consisting of common foods (carbohydrate/protein/fat distribution 55%/15%/30%) were ingested in the General Clinical Research Center. Basal insulin (glargine) taken the night before was adjusted to achieve fasting plasma glucose levels of 3.9–11.1 mmol/l (70–200 mg/dL) the following morning. On each study day, regular insulin was given subcutaneously just before the meal at a dose 30% lower than usual for each patient's rapid analogue for such a meal. The study drug was given (by separate injection, single-masked, in random order) as pramlintide in ratios of 6, 9 or 12 mcg/unit insulin, or as placebo solution, volume-matched (0.015 ml) for the pramlintide 9 mcg/U dose. Blood samples drawn at –15, –5, 15, 30, 45, 60, 90, 120, 150 and 180 min relative to starting the meal were assayed for glucose (Glucose-quant; Roche Diagnostics, Indianapolis, IN, USA), immunoreactive glucagon (glucagon radioimmunoassay; EMD Millipore, Billerica, MA, USA) [8], and pramlintide (enzyme-linked immunosorbent assay; AstraZeneca, unpublished results). The primary endpoint was the 0–3-h incremental area under the curve (AUC_{0-3h}) from baseline of plasma glucose concentrations.

Efficacy, pharmacokinetic (PK) and safety analyses were conducted on the intention-to-treat (ITT), PK and safety analysis sets, respectively. The ITT and safety analysis sets included all randomized patients. One patient was excluded

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from the PK analysis as a result of predose values of plasma pramlintide above the lower limit of quantification in all three pramlintide-containing periods, apparently reflecting occasional high background values with this method. Postprandial incremental AUC_{0-3h} and other continuous efficacy variables were analysed using a linear mixed-effects model with factors for randomized treatment sequence, period and treatment, as well as a random intercept for patient. All treatment effect tests (active vs placebo) were conducted at a two-sided significance level of 0.05, with no adjustment for multiplicity. Adverse events were recorded for the on- and off-treatment periods.

Results

The mean (\pm standard deviation (s.d.)) age of the 19 patients (7 women, 12 men) was 46 ± 16 years, their mean \pm s.d. weight was 81.5 ± 11.1 kg, their mean \pm s.d. body mass index was 26.4 ± 2.6 kg/m², their mean \pm s.d. glycated haemoglobin was $7.75 \pm 0.58\%$ (61 ± 6.3 mmol/mol), and their mean \pm s.d. fasting C-peptide level was 0.016 ± 0.015 nmol/l (normal range 0.2–0.7 nmol/l). A total of 17 patients completed all studies. The mean \pm s.d. fasting plasma glucose before the test meal was 8.4 ± 1.8 mmol/l (152 ± 33 mg/dl). Mean \pm s.d. doses of regular insulin before test meals were 5.4 ± 1.3 , 5.1 ± 1.5 , 5.3 ± 1.4 and 5.1 ± 1.5 units for the 6, 9 and 12 mcg/U and placebo/U studies, respectively. Mean \pm s.d. pramlintide doses were 32 ± 8 , 46 ± 14 and 63 ± 17 mcg for the three ratios, respectively.

Figure 1A–C shows the responses of plasma glucose, glucagon and pramlintide. Compared with placebo/insulin, all three pramlintide/insulin ratios produced marked and statistically equivalent reductions of increments of glucose and glucagon in the first hour, with levels subsequently increasing. After 2 h, glucagon levels for all ratios were similar to placebo, and after 3 h, glucose levels did not differ between groups. The AUC_{0-3h} for glucose was reduced by 60, 58 and 72% for the 6, 9 and 12 mcg/U ratios (Table 1; all doses vs placebo: $p < 0.001$). Reductions of AUC_{0-3h} for glucagon for the 6, 9 and 12 mcg/U were 57, 59 and 55% (Table 1; all doses vs placebo: $p < 0.05$). Pramlintide levels peaked at 15–30 min in a ratio-dependent fashion, with longer persistence of elevation at 12 mcg/U. No symptomatic hypoglycaemia occurred during the 24-h period after pramlintide administration. Between test meal days, two patients reported three events of mild, asymptomatic hypoglycaemia. One patient experienced hypoglycaemia 3 days after placebo administration, and another experienced hypoglycaemia 5 and 7 days after receiving pramlintide 12 mcg/U. Five adverse events were reported. One patient reported mild nausea accompanying each pramlintide/insulin ratio but was able to complete each test meal. Diarrhoea and abdominal pain of severe intensity, considered unrelated to treatment, were reported by one patient.

Conclusions

This proof-of-concept study examined the short-term safety and efficacy of three ratios of pramlintide co-administered with

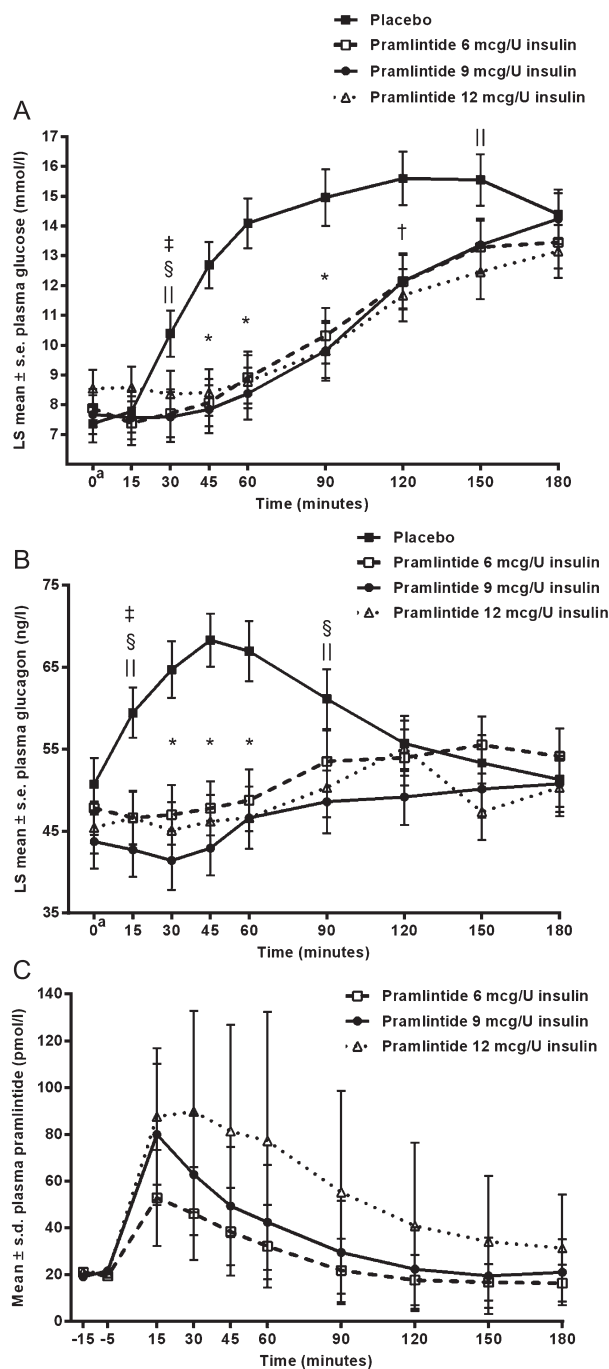


Figure 1. (A) Postprandial glucose, (B) glucagon and (C) pramlintide concentrations over time for each treatment group. Black squares, solid line = placebo + insulin; white squares, dashed line = pramlintide 6 mcg/U insulin; black circles, solid line = pramlintide 9 mcg/U insulin; white triangles, dotted line = pramlintide 12 mcg/U insulin. LS, least squares; s.d., standard deviation; s.e., standard error. ^aTime point 0 represents predose, defined at each visit as the average of values collected <30 min before dosing. Comparisons versus placebo: * $p < 0.001$ (all pramlintide doses); † $p < 0.01$ (all pramlintide doses); ‡ $p < 0.01$ (pramlintide 6 mcg/U insulin); § $p < 0.01$ (pramlintide 9 mcg/U insulin); || $p < 0.05$ (pramlintide 12 mcg/U insulin).

Table 1. Glucose and glucagon incremental AUC_{0–3h}.

Variable	Dose/U insulin	LS mean ± s.e.	Treatment difference	LS mean difference	p
Glucose incremental AUC _{0–3h} (mg × h/dl)	Placebo	343.08 ± 29.94	6–9 mcg	–6.04	0.890
	6 mcg	137.11 ± 30.83	6–12 mcg	40.63	0.343
	9 mcg	143.15 ± 31.08	6 mcg–placebo	–205.98	<0.001
	12 mcg	96.48 ± 29.96	9–12 mcg	46.67	0.280
			9 mcg–placebo	–199.93	<0.001
Glucagon incremental AUC _{0–3h} (pg × h/ml)	Placebo	25.05 ± 5.57	12 mcg–placebo	–246.60	<0.001
			6–9 mcg	0.57	0.934
	6 mcg	10.82 ± 5.71	6–12 mcg	–0.47	0.945
	9 mcg	10.25 ± 5.75	6 mcg–placebo	–14.23	0.041
	12 mcg	11.30 ± 5.56	9–12 mcg	–1.05	0.879
			9 mcg–placebo	–14.80	0.034
			12 mcg–placebo	–13.75	0.046

AUC_{0–3h}, 0–3-h incremental area under the curve; LS, least squares; s.e., standard error.

regular insulin before a standard meal to guide the design of future studies. The patients were experienced in basal-bolus treatment and using moderate prandial doses of a rapid-acting insulin analogue. Regular insulin was used for the test meal in this study because it was previously shown to better match the profile of action needed when gastric emptying is slowed by pramlintide [7]. The ratios tested were chosen based on previous clinical experience with pramlintide [5–7,9] and *in silico* modelling of data from a previous meal study [10]. Insulin dosage was reduced by 30% from usual for each patient to limit the risk of hypoglycaemia. Under these conditions, all ratios markedly reduced increases of both glucose and glucagon during the 3-h follow-up period, without causing hypoglycaemia. Reductions of this magnitude (>50%) are difficult to attain safely with increased dosage of prandial insulin alone. The similarity of reductions between ratios suggests that the pramlintide doses delivered, which generally were in the 30–60-mcg range, were all close to maximally effective in slowing gastric emptying and suppressing glucagon secretion in the first 2 h. All ratios were well tolerated.

These findings are consistent with previous experience, are reassuring regarding safety and provide the basis for future studies; however, interpretation of the potential for this treatment strategy is limited by the use of a single meal-time injection with reduced dosage, and observation for only 3 h after the meal. Glucagon and glucose levels rose after 2–3 h, and this increase might, in theory, be prevented by the use of usual meal-time insulin dosage, together with provision of stable basal levels of pramlintide and insulin in a fixed ratio. Furthermore, this study does not provide insight into the risk of nausea or hypoglycaemia during continuous basal-bolus administration of a fixed ratio, although nausea associated with pramlintide is reported to decline with continued use. Additionally, the long-term potential for this treatment strategy will be influenced by whether a stable co-formulation is possible, as well as by costs; thus, the present findings must be considered preliminary. In conclusion, all pramlintide/insulin ratios effectively and safely reduced glycaemic excursions and suppressed glucagon secretion in the immediate postprandial state. We consider these results to justify longer-term studies of one of these fixed ratios.

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Conflict of Interest

M. C. R. reports receiving honoraria for consulting from AstraZeneca, Bidel, Elcelyx, Eli Lilly, Sanofi and Valeritas; honoraria for lectures from Sanofi; and research grant support through Oregon Health & Science University from AstraZeneca, Eli Lilly, NovoNordisk, Pfizer/Merck, and Sanofi. These dualities of interest have been reviewed and managed by Oregon Health & Science University. T. W. d. B., K. H., P. Ö. and J. X. are or were employees of AstraZeneca. O. G. K. was an employee of Amylin Pharmaceuticals, LLC and has received honoraria for consulting from AstraZeneca. K. C. J. Y. has nothing to declare.

M. C. R. participated in the conception and design of the study, acquisition, analysis and interpretation of data and wrote the manuscript. M. C. R. is the guarantor of this work and,

as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. T. W. d. B. participated in the acquisition, analysis, and interpretation of data and critically revised the manuscript. K. H. participated in the conception and design of the study, interpretation of data and critically revised the manuscript. P. Ö. participated in the interpretation of data and critically revised the manuscript. J. X. participated in the analysis and interpretation of data and critically revised the manuscript. O. G. K. participated in the conception and design of the study, analysis and interpretation of data and critically revised the manuscript. K. C. J. Y. participated in the acquisition and interpretation of data and critically revised the manuscript. All authors approved the final version of the manuscript and agree to be responsible for the accuracy and integrity of the work.

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