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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-332

Clinical Pharmacology and Biopharmaceutics Review

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
REVIEW on AMENDMENT

NDA:	21-332
Submission Date(s):	September 17, 2004
Brand Name:	Symlin™
Generic Name:	Pramlintide acetate for injection
Reviewer:	Sang M. Chung, Ph. D.
Team Leader:	Hae-Young Ahn, Ph. D.
OCPB Division:	Division of Pharmaceutical Evaluation 2 (DPE-2, HFD-870)
OND division:	Division of Metabolic and Endocrine (DMEDP, HFD-510)
Sponsor:	Amylin Pharmaceutics, Inc.
Submission Type:	Response to AE letter dated on December 17, 2003
Indication:	Type 1 or type 2 diabetes mellitus as an adjunctive to insulin

1 Executive Summary

Pramlintide (M.W. 3949.4) is an analogue of human amylin. Pramlintide is reported to delay gastric emptying, reduce glucagons release, and reduce food intake. The effects seem to be via central and vagus nerve systems. The proposed indication is an adjunctive therapy to insulin in patients with type 1 or type 2 diabetes mellitus.

Pramlintide is proposed to be administered by subcutaneous injection immediately prior to each major meal. The proposed dosing is dose escalation for type 2 diabetes from 60 mcg up to 120 mcg with insulin dose adjustment to optimize glycemic control. For type 1 diabetes, the proposed starting dose of Symlin™ is 15 mcg, and dose titration up to 30 mcg or 60 mcg in 15 mcg increments is proposed with insulin dose adjustment to optimize glycemic control. The dose adjustments of Symlin™ and insulin for both types of diabetes are to be made only by the healthy care professionals. Pramlintide will be provided as 0.6 mg/ml in 5 ml vials for use with a syringe. C

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The sponsor received the second approvable letter (December 17, 2003) from the Agency with several deficiencies including the following Clinical Pharmacology and Biopharmaceutics issues (Note: These CPB comments were not approvability issues.):

(*excerption from the letter*)

A. The primary pharmacodynamic (PD) endpoint was the plasma glucose concentration-time curve from time zero to last time point ($AUC_{0\text{-}4hr}$). However, AUC was not an optimal PD endpoint because of the averaging nature between below and above the baseline (fasting glucose level).

B. The abdomen, arm, and thigh were proposed as injection sites. The exposure after injection into the thigh was not different from exposure after injection into the abdomen. However, for obese patients, pramlintide exposure as AUC was 20-36% higher after injection into the arm compared to injection into the abdomen. Therefore, for obese patients, the arm should only be cautiously recommended as an alternative injection site because of considerations of further increased risk of hypoglycemia due to the higher exposure.

There was no new study included in this submission for the CPB issues. However, the sponsor addressed the issues using the cross study comparison.

For the first issue in the letter, the sponsor presented summary of postprandial glucose concentrations-time profiles (e.g., Figure 1). However, the sponsor did not provide an appropriate PD parameter(s) related to the area under the plasma glucose concentration-time profile (AUC_{glu}) reflecting clinical consequences.

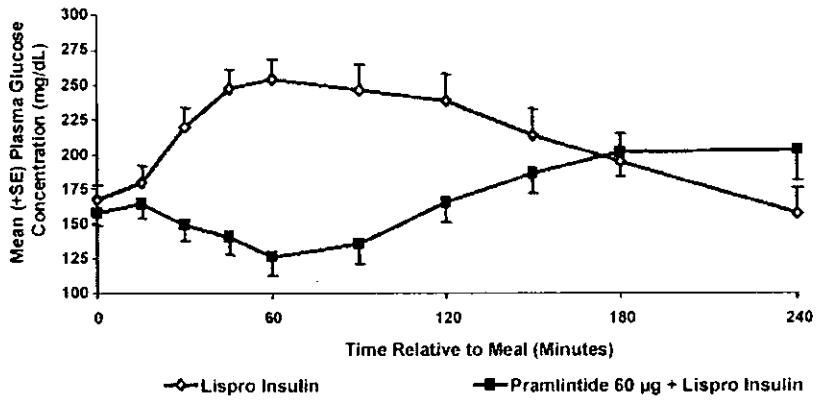

Figure 1 **Mean (n=20) plasma glucose concentrations in type 1 diabetic patients using lispro insulin and pramlintide 60mcg**

In the original NDA, AUC_{glu} was a primary PD parameter, and calculated from time zero to the last sampling (i.e., 4 hours) though the glucose concentrations were below and above the baseline during the samplings. It indicated that AUC_{glu} did not differentiate between AUC under the baseline, a quantitative predictor for hypoglycemic events in conjunction with C_{min}, and AUC above the baseline, a predictor for efficacy. Furthermore, concentrations below the baseline provided false positive for the effect of lowering postprandial glucose excursion without reflecting hypoglycemic events in the original NDA. In conclusion, $AUC_{glu,\ 0\text{-}4hr}$ was not a meaningful primary PD parameter for pramlintide responses because of averaging nature. Therefore, it was recommended

to optimize $AUC_{glu,0\text{-}4hr}$ for the better understanding of the kinetic of drug action. One of the options will be differentiation between AUC_{glu} under the baseline and above the baseline separately (Figure 2) in the future application.

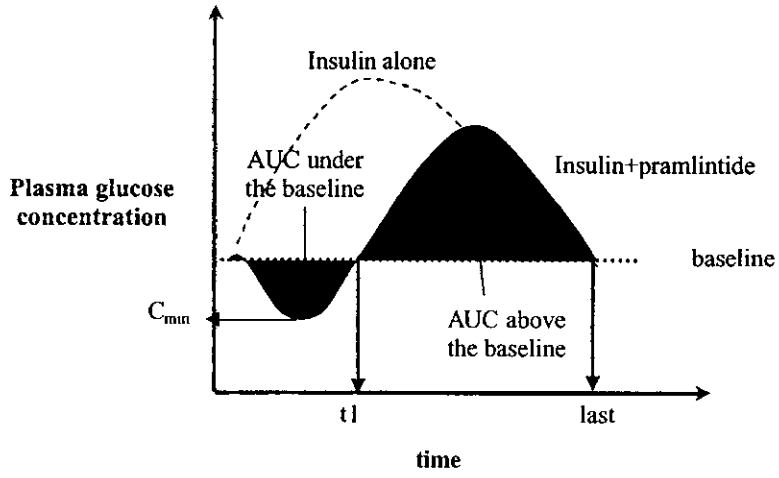

Figure 2 **Schematic summary of proposed optimization of AUC_{glu} as PD parameter.**

For the second issue in the letter, the sponsor proposes to limit the injection sites in the abdomen and thigh area.

1.1 Recommendation

The Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II (OCPB/DPE-2) reviewed the amendment and finds it acceptable. This recommendation and labeling comments should be sent to the sponsor as appropriate.

2 Labeling Comments

(~~Strikethrough~~ indicates deletion and underlined text indicates addition. Labeling claims based on SYMLIN activity in the section of special population (in red) are pending upon clarification on the results).

Pharmacokinetics

Absorption. The absolute bioavailability of a single SC dose of SYMLIN is approximately 30-40%. Subcutaneous administration of different doses of SYMLIN into the abdominal area or thigh of healthy subjects resulted in dose-proportionate for C_{max} and AUC; [ ] (**Table 1**). [

Table 1: Mean Pharmacokinetic Parameters Following Administration of Single SC Doses of SYMLIN

SC Dose (μg)	AUC $_{(0-\infty)}$ (pmol*min/L)	Cmax (pmol/L)	Tmax (min)	Elimination $t_{½}$ (min)
30	3750	39	21	55
60	6778	79	20	49
90	8507	102	19	51
120	11970	147	21	48

[ ] Injection into the arm showed higher exposure with greater variability compared to exposure after injection into the abdominal area or thigh.

There was no strong correlation between the degree of adiposity as assessed by BMI or skin fold thickness measurements and relative bioavailability. Injections administered with 6.0 mm and 12.7 mm needles yielded similar bioavailability.

Distribution. SYMLIN [ ] (approximately 40% of the drug is unbound in plasma), and thus should be insensitive to changes in binding sites.

Metabolism and Elimination. In healthy subjects, the half-life of SYMLIN is approximately 48 minutes. SYMLIN is metabolized primarily by the kidneys. Des-lys[1] pramlintide (2-37 pramlintide), the primary metabolite, has a similar half-life and is biologically active both *in vitro* and *in vivo* in rats.

[ ]

Special Populations.

Renal Insufficiency: Subjects with moderate or severe renal impairment [ ] did not show increased SYMLIN exposure or reduced SYMLIN clearance, compared with subjects with normal renal function. No studies have been done in dialysis patients.

Hepatic Insufficiency: Pharmacokinetic studies have not been conducted in patients with hepatic insufficiency. However, based on the large degree of renal metabolism (see Metabolism and Elimination), hepatic dysfunction is not expected to affect blood concentrations of SYMLIN.

Geriatric: Pharmacokinetic studies have not been conducted in the geriatric population. [

] SYMLIN should only be used in patients known to be able to fully understand and adhere to proper insulin adjustments and glucose monitoring no age-related differences in the activity of SYMLIN have been observed in the geriatric population (N=539 patients 65 years of age or older in the clinical trials).

Pediatric: SYMLIN has not been evaluated in the pediatric population.

Gender: A study has not been conducted to evaluate the gender effect on SYMLIN pharmacokinetics. However, [

] no gender-related differences in the activity of SYMLIN have been observed in the clinical trials (n=2799 for male, and n=2085 for female).

Race/Ethnicity: study has not been conducted to evaluate the effect of ethnicity on SYMLIN pharmacokinetics. However, [

] no differences in the activity of SYMLIN have been observed among patients of differing race/ethnicity in the clinical trials (n=4257 for white, n=229 for black, n=337 for Hispanic, and n=61 for other ethnic origins).

[

]

Drug Interactions

The effect of pramlintide (120mcg) on acetaminophen (1000mg) pharmacokinetics as a gastric emptying marker was evaluated (n=24) in type 2 diabetic patients. Pramlintide did not alter significantly AUC of acetaminophen. However, pramlintide decreased acetaminophen [

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_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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/s/

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2/3/05 02:14:18 PM
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OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
REVIEW on AMENDMENT

NDA:	21-332
Submission Date(s):	June 16, 2003
Brand Name:	Symlin™
Generic Name:	Pramlintide acetate for injection
Reviewer:	Sang M. Chung, Ph. D.
Team Leader:	Hae-Young Ahn, Ph. D.
OCPB Division:	Division of Pharmaceutical Evaluation 2 (DPE-2, HFD-870)
OND division:	Division of Metabolic and Endocrine (DMEDP, HFD-510)
Sponsor:	Amylin Pharmaceutics, Inc.
Submission Type:	1S
Indication:	Type 1 or type 2 diabetes mellitus as an adjunctive to insulin

1 Executive Summary

Pramlintide (M.W. 3949.4) is an analogue of human amylin. Amylin is a 37-amino acid protein co-secreted with insulin from the pancreatic beta-cells. Pramlintide is different from the amylin in 3 amino acids: alanine (position 25), and serine (position 28), and serine (position 29) of amylin are replaced with proline in pramlintide. The change of three amino acids is reported to provide improvement in terms of stability, solubility, non-adhesiveness, and non-aggregation compared to those in amylin. Pramlintide is reported to delay gastric emptying and the effects seem to be via central and vagus nerve systems. The proposed indication is an adjunctive therapy to insulin in patients with type 1 or type 2 diabetes mellitus.

Pramlintide is proposed to be administered by subcutaneous injection immediately prior to each major meal. The proposed dosing is dose escalation for type 1 diabetes and 120 mcg for type 2 diabetes. The proposed starting dose of Symlin™ is 15 mcg and dose titration up to 60 mcg in 15 mcg increments is proposed for type 1 diabetes. Pramlintide will be provided as a 0.6 mg/ml in 5 ml vials for use with a syringe. [redacted]

The sponsor received an approvable letter (October 10, 2001) by the Agency with several deficiencies including the following Clinical Pharmacology and Biopharmaceutics issues: (*excerption from the letter*)

- The reduced bioavailability of pramlintide observed in patients with Type 2 diabetes relative to those with Type 1 diabetes supports differential dosing between the two populations. The difference in bioavailability may be related to skin thickness, amount of subcutaneous fat, and/or site of injection. A clearer understanding of the mechanism(s) responsible for the observed difference is necessary to guide the safe use of the drug (e.g., in lean patients with Type 2 diabetes).
- The optimally safe and effective timing of Symlin™ and insulin administration relative to food ingestion has not been established.
- The effect of concomitant Symlin™ administration on the pharmacokinetics of orally administered drugs requires further characterization.

To explore the issues, the sponsor conducted three pharmacokinetic studies and concluded as follows:

- There was no statistical difference in relative bioavailability 1) among injection sites (abdomen, arm, and thigh) 2) between needle lengths (6.0-mm and 12.7-mm), and 3) between types of patient (type 1 and type2) (protocol 137-153).
- The optimal timing of dose to the meals was immediately prior to meal ingestion based on blood glucose results (protocol 137-151).
- For drugs that primary efficacy is dependent on rapid absorption and C_{max} (i.e., pain killer), the proposed dosing time window of co-administration was set as at least 1 hour prior to or 3 hours after pramlintide administration. The time window was proposed based on the effect of pramlintide on oral acetaminophen (protocol 137-154).

There are several major comments related to Clinical Pharmacology and Biopharmaceutics as follow:

- The type 1 obese patients showed lower pramlintide exposure compared to that in other patient groups (i.e., type 1 non-obese, type 2 obese, and type 2 non-obese). In the original NDA, the pramlintide exposure in type 2 diabetes appeared to be lower than that in type 1 diabetes according to the observations. In this regard, the relative bioavailability difference between types of diabetic patient was not conclusive.
- The abdomen, arm and thigh were proposed as injection sites. The exposure after injection into thigh was not different from that after in abdomen. However, pramlintide exposure as AUC was 20-36% higher after arm injection compared to that in abdomen for obese patients. Therefore, arm should be cautiously considered as an alternative injection site for obese patients because of hypoglycemic safety concern with the higher exposure.
- The primary pharmacodynamic endpoint was the plasma glucose concentration-time curve from time zero to last time point ($AUC_{0\text{-}4hr}$). However, the AUC was not an optimal PD endpoint because of averaging nature between below and above the baseline (fasting glucose level).
- Inhibition of postprandial glucose excursion was one of the proposed beneficial effects of pramlintide. Mean maximum postprandial glucose elevation ($C_{max,glu}$) was observed as 87.2mg/dL for placebo (insulin alone), 77.7mg/dL for pramlintide dosing 15 minutes before breakfast, and 47.2mg/dL for pramlintide dosing immediate before

breakfast in type 1 using lispro insulin group. However, the elevated glucose levels ($C_{max,glu}$) were observed at the last sampling of the study (i.e., 4 hours after breakfast). The some levels were as close as the maximum postprandial glucose elevations of placebo. In these regards, it was premature to assess the beneficial role of pramlintide to postprandial glucose excursion based on the results in the type 1 using lispro insluin.

1.1 Recommendation

The Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II (OCPB/DPE-2) reviewed the amendment and finds it acceptable provided that appropriate resolutions be made on the comments and labeling changes. This recommendation and comments should be sent to the sponsor as appropriate.

1.2 Phase IV Commitments

N/A

2 Table of Contents

3 Question Based Review (QBR)

Detailed information on pramlintide is available from the original NDA and OCPB review, and brief summary on background related to this amendment will be described in the following QBR.

3.1 General Attributes

Pramlintide acetate is a synthetic analog of human amylin. Amylin is a 37-amino acid polypeptide co-secreted with insulin from the beta-cells of pancreas. Pramlintide is synthesized by replacement with proline at three amino acids in amylin as shown in the following sequence;

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-*Pro*-Ile-Leu-*Pro-Pro*-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH_2 acetate (salt) with a disulfide bridge between the two Cys residues.

The amino acid change is known to provide favorable physicochemical properties to the pramlintide compared to those in amylin. Pramlintide acetate is a white powder and soluble in water.

3.2 General Clinical Pharmacology

Amylin appears to be a neuroendocrine hormone and primarily regulates blood glucose by slowing gastric emptying and inhibiting postprandial glucagon secretion. The effect of amylin on gastric emptying has been proposed through central mechanism. Pramlintide is expected to control postprandial glucose control as an adjunctive to insulin.

It is reported that the fasting amylin levels are at the lower end of the available assay range (4-8 pmol/L) and increased several fold from the baseline following meal ingestion.

3.3 Intrinsic Factors

3.3.1 What are the effects of injection sites (abdomen, arm, and thigh), needle lengths (6.0-mm and 12.6-mm), and obesity in type 1 and type 2 on pramlintide bioavailability?

Pramlintide exposure was assessed in a randomized, open-label, 4-way crossover study in non-obese and obese patients with type 1 and type 2 diabetes (Protocol No. 137-15). Patients were randomized to one of 4 treatments (Treatment A to D), and intravenous administration (Treatment E) was followed other 4 treatments at the final day (Day 5). Treatments were as follows:

Study Treatments

- Treatment A: Abdomen (SC) using 6.0-mm needle
- Treatment B: Abdomen (SC) using 12.7-mm needle
- Treatment C: Arm (SC) using 12.7-mm needle
- Treatment D: Thigh (SC) using 12.7-mm needle
- Treatment E: IV bolus

Pramlintide was administered immediately prior to breakfast. Doses were 60 mcg for type 1 diabetes, 120 mcg for type 2 diabetes, and 20 mcg for intravenous administration regardless of types of patient. There were 4 study groups as follows:

Parallel study with 4 groups

Study Group 1: non-obese subjects with type 1 diabetes (BMI $\leq$ 27 kg/m^2)
Study Group 2: obese subjects with type 1 diabetes (BMI 30 kg/m^2 to 45 kg/m^2)
Study Group 3: non-obese subjects with type 2 diabetes (BMI $\leq$ 27 kg/m^2)
Study Group 4: obese subjects with type 2 diabetes (BMI 30 kg/m^2 to 45 kg/m^2)

The numbers of patients in the study are summarized in the following table. Conclusions were based on the evaluable data and the sponsor sometimes excluded patients showing significantly lower exposure possibly due to dosing error.

Table 1 **The number of subjects by intent-to-treat (ITT), per protocol (PPT), evaluable, and PK analyses among 75 subjects who enrolled for the study.**

	ITT	PPT	Evaluable	PK analyses
Type 1 non-obese	20	19	18	16
Type 1 obese	15	15	15	15
Type 2 non-obese	17	17	16	14
Type 2 obese	18	18	18	18
Total	70	69	67	63

Plasma samples were obtained to measure pramlintide and the immunoenzymetric assay was used to measure pramlintide.

Pharmacokinetic parameters are summarized in the following table.

Table 2 **Mean (SD) plasma pramlintide pharmacokinetic parameters**

Mean (SD) Plasma Pramlintide $AUC_{(0-4\ h)}$ and C_{max} by Treatment and Study Group
Population: Evaluable (N=67)

Type 1 Non-obese (n=18) Pramlintide SC 60 µg				
	Abdomen 12.7-mm	**Abdomen 6.0-mm**	**Arm 12.7-mm**	**Thigh 12.7-mm**
AUC(0-4 h) (pmol*h/L)				
Mean (SD)	132.6 (70.7)	117.6 (56.5)	144.7 (82.3)	135.5 (81.2)
CV	53.3	48.1	56.8	59.9
Cmax (pmol/L)				
Mean (SD)	107.4 (46.2)	105.1 (32.8)	95.0 (49.4)	82.1 (39.9)
CV	43.0	31.2	52.0	48.7
Type 1 Obese (n=15) Pramlintide SC 60 µg				
	Abdomen 12.7-mm	**Abdomen 6.0-mm**	**Arm 12.7-mm**	**Thigh 12.7-mm**
AUC(0-4 h) (pmol*h/L)				
Mean (SD)	88.6 (57.0)	87.7 (46.9)	122.9 (89.4)	91.1 (46.7)
CV	64.3	53.5	72.8	51.2
Cmax (pmol/L)				
Mean (SD)	66.6 (26.7)	73.4 (22.8)	85.8 (38.9)	58.5 (23.3)
CV	40.1	31.1	45.3	39.8
Type 2 Non-obese (n=16) Pramlintide SC 120 µg				
	Abdomen 12.7-mm	**Abdomen 6.0-mm**	**Arm 12.7-mm**	**Thigh 12.7-mm**
AUC(0-4 h) (pmol*h/L)				
Mean (SD)	281.1 (195.5)	201.7 (80.3)	273.6 (143.5)	240.4 (130.0)
CV	69.5	39.8	52.5	54.1
Cmax (pmol/L)				
Mean (SD)	167.6 (53.2)	167.6 (53.2)	166.0 (71.6)	127.4 (72.2)
CV	31.7	31.7	43.1	56.6
Type 2 Obese (n=18) Pramlintide SC 120 µg				
	Abdomen 12.7-mm	**Abdomen 6.0-mm**	**Arm 12.7-mm**	**Thigh 12.7-mm**
AUC(0-4 h) (pmol*h/L)				
Mean (SD)	266.3 (260.5)	279.2 (242.5)	367.0 (387.7)	289.0 (281.4)
CV	97.8	86.8	105.6	97.4
Cmax (pmol/L)				
Mean (SD)	185.3 (143.3)	184.5 (154.1)	237.5 (223.0)	151.1 (114.0)
CV	77.3	83.5	93.9	75.5

The exposure after pramlintide injection into thigh was not significantly different from that after abdomen injection within the each study groups. However, injection into arm showed higher exposure compared to abdomen injection in obese patients. In non-obese patients, all three injection sites showed similar exposure with the same needle length. In this regard, arm should be cautiously considered as an alternative injection site because of hypoglycemic safety concern with the higher exposure.

There was no significant difference in exposure between different needle lengths (6.0-mm vs. 12.7-mm). Results of relative bioavailability to abdomen as a reference are summarized in the following table.

Table 3 **Relative bioavailability to abdomen 12.7-mm needle length as a reference.**

Relative Bioavailability of Pramlintide by Treatment by Study Group
Population: Evaluable (N=67)

Type 1 Non-obese (n=18) Pramlintide SC 60 µg				
	Abdomen 12.7-mm	Abdomen 6.0-mm	Arm 12.7-mm	Thigh 12.7-mm
AUC(0–4 h) (pmol*h/L)				
Geometric LS Mean Ratio (SE)	reference	0.92 (0.10)	1.09 (0.11)	1.02 (0.11)
Geometric 90% C I	reference	(0.77, 1.09)	(0.91, 1.30)	(0.86, 1.22)
Cmax (pmol/L)				
Geometric LS Mean Ratio (SE)	reference	1.01 (0.11)	0.84 (0.09)	0.75 (0.08)
Geometric 90% C I	reference	(0.84, 1.21)	(0.70, 1.02)	(0.62, 0.90)
Type 1 Obese (n 15) Pramlintide SC 60 µg				
	Abdomen 12.7-mm	Abdomen 6.0-mm	Arm 12.7-mm	Thigh 12.7-mm
AUC(0–4 h) (pmol*h/L)				
Geometric LS Mean Ratio (SE)	reference	1.03 (0.11)	1.36 (0.15)	1.06 (0.11)
Geometric 90% C I	reference	(0.86, 1.23)	(1.13, 1.63)	(0.89, 1.27)
Cmax (pmol/L)				
Geometric LS Mean Ratio (SE)	reference	1.14 (0.11)	1.29 (0.13)	0.89 (0.09)
Geometric 90% C I	reference	(0.96, 1.35)	(1.09, 1.53)	(0.75, 1.05)
Type 2 Non-obese (n=16) Pramlintide SC 120 µg				
	Abdomen 12.7-mm	Abdomen 6.0-mm	Arm 12.7-mm	Thigh 12.7-mm
AUC(0–4 h) (pmol*h/L)				
Geometric LS Mean Ratio (SE)	reference	0.81 (0.10)	1.03 (0.12)	0.91 (0.11)
Geometric 90% C I	reference	(0.67, 0.99)	(0.84, 1.26)	(0.75, 1.12)
Cmax (pmol/L)				
Geometric LS Mean Ratio (SE)	reference	0.89 (0.11)	0.83 (0.11)	0.61 (0.08)
Geometric 90% C I	reference	(0.72, 1.10)	(0.67, 1.03)	(0.49, 0.76)
Type 2 Obese (n 18) Pramlintide SC 120 µg				
	Abdomen 12.7-mm	Abdomen 6.0-mm	Arm 12.7-mm	Thigh 12.7-mm
AUC(0–4 h) (pmol*h/L)				
Geometric LS Mean Ratio (SE)	reference	1.06 (0.10)	1.20 (0.11)	0.99 (0.09)
Geometric 90% C I	reference	(0.90, 1.24)	(1.03, 1.41)	(0.85, 1.16)
Cmax (pmol/L)				
Geometric LS Mean Ratio (SE)	reference	0.97 (0.10)	1.13 (0.12)	0.76 (0.08)
Geometric 90% C I	reference	(0.81, 1.15)	(0.95, 1.35)	(0.64, 0.91)

There was exposure difference in type 1 obese diabetes compared to that in other groups. The pramlintide exposure in type 1 obese diabetes was about 62-72% to other patient subsets based on dose normalized ratios of mean AUC and C_{max} in the proposed injection site and needle length (i.e., abdomen injection with 12.7-mm needle length). These results were different from the observation in the original NDA. According to the review of the original NDA, the exposure tended to be lower in type 2 compared to that in type 1 patients (refer the results from the original NDA review in the following table). Therefore, the results on relative bioavailability difference in types of diabetic patients are inconclusive and need to be elucidated further.

Table 4 **PK results from the review of the original NDA (Study 137-143 and -144)**

		Type 1 (N=11)		Type2 (N=12)	
Dose	Parameters	Group 1	Group 2	Group1	Group2
60 mcg	AUC_{0-t} (pmol*hr/L)	74.14±34.26	51.67±33.37	62.39±61.98	32.24±46.23
	C_{max} (pmol/L)	64.5±23.7	66.9±25.0	50.8±20.3	36.4±18.5
90 mcg	AUC_{0-t} (pmol*hr/L)	118.5±65.37	115.9±5429		53.93±52.15
	C_{max} (pmol/L)	99.4±31.4	123.9±40.5		55.7±25.7
120 mcg	AUC_{0-t} (pmol*hr/L)			143.0±139.9	76.24±54.37
	C_{max} (pmol/L)			117.4±116.4	74.0±24.6

Intersubject variability was high in the exposure data and thus may provide the above inconsistent results. In these regards, this reviewer explored the following two factors to minimize the sources of intersubject variability in PK characterization.

- Patient population

Patient population in the study appeared to be heterogeneous and it may add variability to the results. The sub-populations based on gender and ethnicity are summarized in the following table. In general, the number and relative ratio of each sub-population varied across the study groups. In addition, the number of patients was not large enough for statistical power in most sub-populations. Therefore, it was not feasible to do exposure comparison based on a homogenous population.

Table 5 **Demographic summary on gender and ethnicity**

Study Group		Black	Caucasian	Hispanic
Type 1 non-obese	Female	2	6	0
	Male	0	8	0
Type 1 obese	Female	6	4	1
	Male	0	4	0
Type 2 non-obese	Female	4	4	0
	Male	4	2	0
Type 2 obese	Female	6	3	1
	Male	1	6	1

- Absolute bioavailability

Absolute bioavailability may provide reduced intersubject variability in PK analyses. Individual data were submitted in response to request by the reviewer and the summary of absolute bioavailability is shown in the following table. The sponsor concluded the results were not definitive because the data showed large variability that maybe be related to small volumes required for IV administration.

Table 6 **Absolute bioavailability**

	N	Treatment	A	B	C	D
Type 1 non-obese	**16**	**Mean**	**0.910**	**0.960**	**1.044**	**0.963**
		SD	0.941	0.892	0.917	0.965
		CV	103	93	88	100
Type 1 obese	**15**	**Mean**	**0.448**	**0.462**	**0.739**	**0.475**
		SD	0.377	0.446	0.857	0.409
		CV	84	96	116	86
Type 2 non-obese	**14**	**Mean**	**0.423**	**0.576**	**0.622**	**0.524**
		SD	0.473	0.725	0.811	0.604
		CV	117	126	130	115
Type 2 obese	**18**	**Mean**	**0.385**	**0.364**	**0.517**	**0.381**
		SD	0.268	0.256	0.472	0.271
		CV	70	70	91	71

Overall for the factors affecting pramlintide bioavailability, the following conclusions made by the sponsor are found to be agreeable:

- There was no significant difference in exposure between two needle lengths (6.0-mm vs. 12.7-mm).
- Thigh may be an alternative injection site with comparable exposure with abdomen injection.

Although the sponsor concluded that arm might be an alternative injection site, injection into arm, however, produced higher exposure in obese patients and higher exposure of pramlintide may be related to hypoglycemic incidents.

In addition, the factor(s) showing lower exposure in the type 1 obese patients need to be further explored. The original NDA showed lower exposure in type 2 compared to that in type 1 diabetes. The sponsor explained that the dosing difference in types of diabetes was based on the patterns of insulin requirement.

3.3.2 What is the effect of pramlintide dose timing to meal on postprandial plasma glucose level?

The effect of pramlintide dosing time on postprandial glucose profiles was assessed in a randomized, single-blind, placebo-controlled, 5-way crossover study with a type 1 and type 2 diabetic patients. Treatments are summarized in the following table.

Table 7 **Treatments**

Treatment	Study Medication	Timing relative to the breakfast
A	Placebo	-15 min
B	Pramlintide	-15 min
C	Pramlintide	0 min
D	Pramlintide	+15 min
E	Pramlintide	+30 min

Pramlintide doses were 60 mcg, and 120 mcg for type 1 and type 2 diabetes, respectively. There were three study groups as follows:

- Study group 1: patients with type 1 diabetes using insulin lispro (n=21; female=8, male=13)
- Study group 2: patients with type 1 diabetes using regular insulin (n=19; female=5, male=14)
- Study group 3: patients with type 2 diabetes using insulin lispro (n=19; female=10, male=9)

Insulin lispro is a rapid-acting human insulin analog (t_{max} is about 30-90 minutes after SC injection) and it is identical to the human insulin except reverse amino acids sequence between 28 and 29 at B-chain. It showed to be equipotent to human insulin on a molar basis. The timing of insulin dose was 0 min and -30 min to the breakfast for short-acting insulin (lispro) and for regular insulin, respectively.

The primary pharmacodynamic (PD) measure was under the plasma glucose concentration-time curve from time zero to last time point ($AUC_{0\text{-}4hr}$) after correction with fasting glucose level. Descriptive comparison of the glucose profiles was also included. In addition, pramlintide PK parameters were estimated using the same method in the previous studies.

Plasma glucose concentration-time profiles after baseline correction are shown in the following figures and the mean (SD) $AUC_{0\text{-}4hr}$ after the treatments are summarized in the following table.

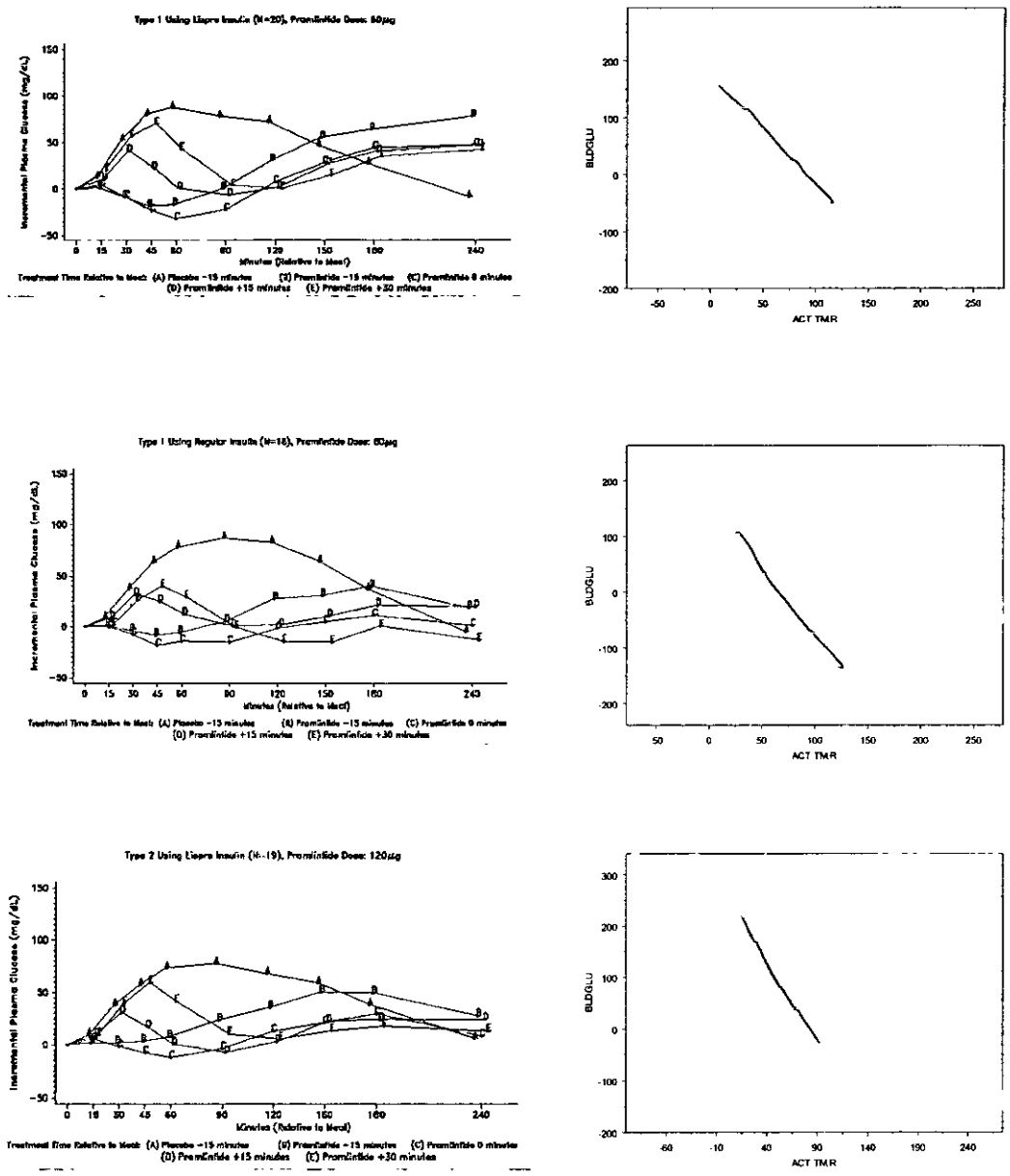

Figure 1 **Plasma glucose concentration-time profiles. Upper panel; type 1 using lispro insulin, middle panel; type 1 using regular insulin, and lower panel; type 2 using lispro insulin. Left panel for mean and right panel for individual with Loess fit line. Treatments are indicated as corresponding letters in the left panel.**

Table 8 **Mean (SD) plasma glucose AUC_{0-4hr}**

Mean (SD) Incremental Plasma Glucose $AUC_{(0-4\,hr)}$ (mg*hr/dL) Following a Standardized Breakfast by Study Group and by Treatment

Evaluable Type 1 Subjects Using Insulin Lispro (N=20)				
Placebo	**Pramlintide 60 µg**			
-15 min	-15 min	0 min	+15 min	+30 min
179.1 (198.9)	114.4 (186.6)	45.1 (169.2)	81.7 (218.4)	109.6 (164.4)
Evaluable Type 1 Subjects Using Regular Insulin (N=18)				
Placebo	**Pramlintide 60 µg**			
-15 min	-15 min	0 min	+15 min	+30 min
198.4 (220.4)	69.0 (189.4)	-10.3 (188.9)	54.0 (184.4)	0.9 (227.2)
Evaluable Type 2 Subjects Using Insulin Lispro (N=19)				
Placebo	**Pramlintide 120 µg**			
-15 min	-15 min	0 min	+15 min	+30 min
187.9 (170.0)	109.9 (148.7)	36.2 (118.0)	51.3 (141.4)	76.8 (171.1)

The sponsor concluded that pramlintide administration immediately before the breakfast observed the largest reduction. However, the parameter does not provide critical insight of efficacy and safety aspects because of averaging nature. In these regards, general conclusions based on the parameter were not acceptable.

Descriptive summary of fasting glucose corrected plasma glucose levels are summarized in the following table.

Table 9 **Mean (SD) plasma glucose concentration-time data with range (2-OCT-2003 submission)**

Time From Breakfast	Placebo	Evaluable Type 1 Subjects Using Insulin Lispro (N=20) Pramlintide 60 µg			
	-15 min	-15 min	0 min	+15 min	+30 min
(min)	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max
30	52.6 (34.6)	-8.2 (17.4)	-8.7 (28.8)	41.1 (31.7)	56.3 (32.6)
45	79.9 (38.9)	**-18.3 (29.6)**	-23.0 (35.3)	22.5 (35.0)	70.3 (40.4)
60	87.2 (46.6)	-16.6 (40.7)	**-31.9 (43.0)**	0.9 (45.6)	42.9 (44.3)
90	78.0 (59.3)	0.3 (60.8)	-21.9 (57.9)	**-6.3 (65.1)**	4.0 (48.3)
120	71.9 (66.2)	30.8 (63.7)	7.5 (61.5)	1.4 (76.5)	**0.5 (51.1)**
150	46.8 (67.4)	55.2 (68.0)	28.3 (56.9)	26.9 (80.4)	14.7 (55.1)
180	27.0 (68.2)	63.8 (79.2)	44.2 (66.6)	40.4 (80.6)	34.8 (59.2)
240	-8.4 (67.1)	77.7 (82.3)	47.2 (80.8)	46.7 (95.8)	41.9 (84.3)

Time From Breakfast	Placebo	Evaluable Type 1 Subjects Using Regular Insulin (N=18) Pramlintide 60 µg			
	-15 min	-15 min	0 min	+15 min	+30 min
	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max
30	37.1 (31.6)	-3.5 (30.1)	-7.4 (15.9)	31.9 (27.1)	26.1 (32.8)
45	63.1 (36.9)	**-8.4 (37.6)**	**-18.5 (24.6)**	25.0 (33.0)	39.8 (37.4)
60	77.8 (48.3)	-5.9 (44.8)	-14.3 (26.0)	12.2 (38.1)	29.3 (49.9)
90	86.9 (60.1)	5.5 (59.1)	-15.1 (48.9)	**0.7 (51.6)**	-0.9 (65.2)
120	82.9 (69.9)	27.6 (60.6)	-1.7 (63.4)	1.7 (62.0)	-14.4 (65.7)
150	64.0 (71.7)	30.7 (60.9)	4.7 (69.1)	10.5 (63.6)	**-15.1 (71.4)**
180	36.8 (76.1)	39.8 (67.8)	11.2 (71.0)	21.2 (66.0)	0.7 (73.8)
240	-5.2 (86.2)	18.6 (66.4)	1.1 (88.6)	19.6 (68.9)	-12.9 (85.4)

	Placebo	Evaluable Type 2 Subjects Using Insulin Lispro (N=19) Pramlintide 120 µg			
	-15 min	-15 min	0 min	+15 min	+30 min
	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max	mean (SD) min, max
30	37.9 (20.6)	**1.7 (16.2)**	-1.6 (15.6)	30.7 (21.9)	39.4 (32.7)
45	57.4 (31.6)	3.3 (25.5)	-7.6 (23.1)	17.0 (27.6)	59.2 (39.1)
60	72.9 (34.9)	7.6 (31.1)	**-12.3 (33.3)**	0.7 (33.7)	41.4 (41.1)
90	77.4 (42.8)	23.4 (39.3)	-3.6 (42.5)	**-7.2 (40.6)**	19.4 (42.5)
120	67.7 (54.3)	35.7 (43.0)	12.8 (40.5)	3.0 (54.8)	**5.7 (54.8)**
150	59.1 (61.1)	50.5 (60.9)	21.3 (38.3)	22.5 (47.0)	13.6 (58.5)
180	38.5 (66.0)	49.7 (72.1)	29.8 (43.3)	24.5 (48.2)	17.9 (60.0)
240	6.1 (63.9)	27.7 (68.9)	8.3 (60.8)	24.1 (52.8)	13.1 (61.5)

Among the treatments, the mean lowest plasma glucose level (-12.3 to -31.9mg/dL) was observed in the treatment C (i.e., pramlintide injection immediately before the breakfast) for the all study groups. From the results, the sponsor proposed the optimal dosing time as the immediately prior to meal ingestion.

There were two issues for the conclusion. The first issue was related to efficacy. The mean maximum postprandial glucose levels ($C_{max,glu}$) were observed at 240 minutes after the breakfast in the treatment B and C as 78±82.3mg/dL and 47±80.8mg/dL, respectively, for type 1 using lispro insulin. The mean $C_{max,glu}$ in placebo was 87±46.6mg/dL at 60 minutes after the breakfast. The $C_{max,glu}$ in treatment B was not significantly different from that in placebo. In addition, the maximum was observed in the last sampling time for the treatment B and C. The results indicated that pramlintide delayed gastric emptying and postpone elevating postprandial glucose levels but did not reduce postprandial glucose levels. Therefore, the results did not show the inhibition effect of pramlintide on postprandial glucose excursion in the type 1 diabetes using lispro insulin. The second issue was about the optimal dosing time. The treatment C showed the most significant reduction of mean postprandial glucose level below the baseline among treatments in the all study groups. The results generated the highest hypoglycemic safety concern to the proposed optimal dosing time. Collectively, the proposed optimal dosing time appeared to be the highest safety concern without or minimal efficacy based on the plasma glucose data. However, it should be noted that inter-subject variability is extremely high and data interpretation using mean data may be caution.

Pramlintide pharmacokinetic results are summarized in the following figures and tables. There was no statistical significant difference in pramlintide PK among treatments.

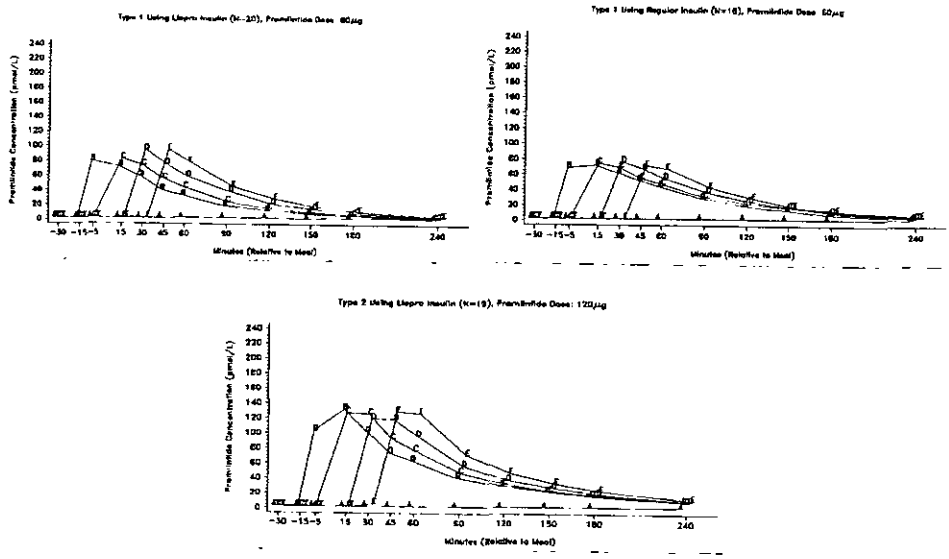

Figure 2 **Plasma pramlintide concentration-time profiles: upper left panel-type 1 using lispro, upper right panel-type 1 using regular insulin, and lower panel-type 2 lispro insulin**

Table 10 **Mean (SD) pramlintide PK parameters**

Parameter	Evaluable Type 1 Subjects Using Insulin Lispro (N=20) Pramlintide 60 μg							
	-15 min		0 min		+15 min		+30 min	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
AUC_{0-4hr}* (pmole·hr/L)	106.4	(47.6)	103.3	(62.4)	113.2	(56.4)	112.5	(74.1)
C_{min} (pmol/L)	25.0	(11.2)	25.8	(15.7)	30.2	(15.2)	32.1	(21.2)
C_{max} (pmol/L)	84.7	(33.7)	89.5	(40.5)	98.2	(41.9)	98.5	(49.6)
T_{max} (hr)	0.3	(0.2)	0.3	(0.1)	0.3	(0.2)	0.3	(0.1)
$t_{1/2}$ (hr)	0.8	(0.2)	0.7	(0.2)	0.8	(0.3)	0.8	(0.2)
	Evaluable Type 1 Subjects Using Regular Insulin (N=18) Pramlintide 60 μg							
	-15 min		0 min		+15 min		+30 min	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
AUC_{0-4hr}* (pmole·hr/L)	125.3	(55.8)	117.6	(83.0)	109.3	(59.0)	103.9	(47.3)
C_{min} (pmol/L)	29.5	(13.2)	29.4	(20.7)	29.2	(15.7)	29.7	(13.6)
C_{max} (pmol/L)	86.8	(27.9)	82.5	(41.1)	82.0	(33.4)	78.2	(30.3)
T_{max} (hr)	0.4	(0.3)	0.4	(0.2)	0.3	(0.3)	0.3	(0.2)
$t_{1/2}$ (hr)	0.8	(0.4)	0.9	(0.5)	0.7	(0.3)	0.8	(0.5)
	Evaluable Type 2 Subjects Using Insulin Lispro (N=19) Pramlintide 120 μg							
	-15 min		0 min		+15 min		+30 min	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
AUC_{0-4hr}* (pmole·hr/L)	200.7	(123.7)	188.5	(135.1)	180.2	(144.6)	182.8	(133.1)
C_{min} (pmol/L)	47.2	(29.3)	47.1	(33.8)	48.0	(38.4)	52.5	(37.9)
C_{max} (pmol/L)	147.3	(89.5)	153.8	(105.7)	135.3	(88.6)	150.4	(130.0)
T_{max} (hr)	0.4	(0.2)	0.4	(0.1)	0.4	(0.2)	0.4	(0.1)
$t_{1/2}$ (hr)	1.1	(0.4)	1.0	(0.3)	0.9	(0.3)	0.9	(0.3)

*AUC_{0-4hr} is the AUC from time of pramlintide injection through 4 hours post-breakfast.

In overall conclusion, pramlintide delayed significantly gastric emptying and thus delayed the maximum postprandial plasma glucose excursion but did not inhibit postprandial glucose excursion in type 1 patients using lispro insulin. The proposed optimal dosing time should be further evaluated because of the inconclusive efficacy and the highest hypoglycemic concern in the treatment C based on plasma glucose data as well as large variability. In addition, the proposed primary pharmacodynamic endpoint (AUC_{0-4hr}) was not acceptable.

3.3.3 What is the effect of pramlintide on other drugs?

Pramlintide is known to delay gastric emptying time and thus it was recommended to assess the effect of pramlintide on pharmacokinetics of other drugs. To address the issue, the sponsor conducted a randomized, single-blind, placebo-controlled, 6-way crossover study using acetaminophen (APAP) with type 2 diabetic patients (n=24; female=9 and male=15). Pramlintide of 120 mcg was administered immediately before breakfast and 1000 mg APAP was administered various time to pramlintide injection as follows:

Treatment	Pramlintide or Placebo	Dosing time of APAP to pramlintide injection
A	Placebo	0 hour
B	Pramlintide 120 mcg	0 hour
C	Pramlintide 120 mcg	-2 hour
D	Pramlintide 120 mcg	-1 hour
E	Pramlintide 120 mcg	+1 hour

F	Pramlintide 120 mcg	+2 hour	

The primary PK parameters were the area under the plasma APAP concentration-time profile ($AUC_{0\text{-}12hr}$) and C_{max}. The results are summarized in the following figure and table.

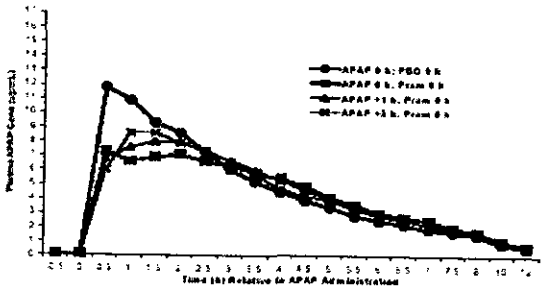

Figure 3 **Mean plasma APAP concentration profiles.**

Table 11 **Mean (SD) plasma acetaminophen PK parameters by treatment groups. (N=24)**

	APAP -2 h PRAM 0 h		APAP -1 h PRAM 0 h		APAP 0 h PBO 0 h		APAP 0 h PRAM 0 h		APAP +1 h PRAM 0 h		APAP +2 h PRAM 0h	
APAP Parameter	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
$AUC_{0\text{-}12h}$ (µg*h/mL)	47.1	(16.2)	47.3	(15.3)	45.2	(15.5)	42.4	(15.4)	43.6	(14.3)	42.8	(15.6)
C_{max} (µg/mL)	15.9	(3.9)	15.7	(4.1)	12.8	(4.6)	9.1	(2.6)	9.9	(3.0)	10.2	(3.5)
T_{max} (h)	0.6	(0.2)	0.5	(0.2)	0.8	(0.4)	2.0	(1.7)	1.6	(1.0)	1.6	(0.9)
$t_{1/2}$ (h)	2.9	(1.9)	2.4	(0.6)	2.5	(0.7)	2.8	(1.1)	2.6	(0.6)	2.6	(0.9)

Note: Treatment columns represent the timing of APAP (1000 mg) administration relative to study medication (pramlintide or placebo) at 0 h.

Acetaminophen has been reported having a gastric emptying limited absorption. According to the results, pramlintide did not alter significantly AUC of APAP. However, pramlintide decreased APAP C_{max} (about 29% at simultaneous co-administration) and increased t_{max} (ranging from 48 to 72 minutes). The increased t_{max} could be from delayed lag time and reduced half-emptying time according to the results in the original NDA (Protocol 137-118) as summarized in the following table.

Table 12 **The effect of pramlintide (15 minutes before the meal) on mean gastric-emptying time in type 1 patients (n=11).**

		Placebo	Pramlintide 30 mcg	Pramlintide 60 mcg	Pramlintide 90 mcg
Breakfast	Half-emptying time (min)	128.6	187.2	200.1	214.5
	Difference from placebo		58.9	69.4	86.3
	Lag time (min)	32.5	54.4	56.4	70.3
	Difference from placebo		21.9	23.9	37.8
Lunch	Half-emptying time (min)	136.9	138.5	140.6	145.4
	Difference from placebo		2.4	2.4	8.8
	Lag time (min)	34.2	46.5	40.0	48.2
	Difference from placebo		12.3	5.8	14

In general, the study results were properly incorporated in the label for drugs of which primary efficacy is dependent on rapid absorption and C_{max} (i.e., pain killer).

However, it should be of caution in the extrapolation of the results to other drugs. Sometimes, gastric emptying affects the extent of oral bioavailability through several factors (i.e., stability in the stomach, absorption window, or first-pass metabolism). Particularly, type 2 diabetic patients can take other oral hypoglycemic drugs and the effects of pramlintide on those should be cautiously interpreted with other confounding factors.

3.4 Extrinsic Factors

3.5 General Biopharmaceutics

[redacted] In vials, each milliliter of SYMLIN contains 0.6 mg of pramlintide (as pramlintide acetate). The common excipients are 2.25 mg of metacresol as a preservative, D-mannitol as a tonicity modifier, and acetic acid and sodium acetate as pH modifiers to obtain a pH of 4.0.

3.6 Analytical

The pramlintide was measured using immunoenzymetric assay (IEMA) [redacted] The assay was acceptable according to the review of the original NDA.

4 Comments

- The type 1 obese patients showed lower pramlintide exposure compared to that in other patient groups (i.e., type 1 non-obese, type 2 obese, and type 2 non-obese). In the original NDA, the pramlintide exposure in type 2 diabetes appeared to be lower than that in type 1 diabetes according to the observations. In this regard, the relative bioavailability difference between types of diabetic patient was not conclusive.
- The abdomen, arm and thigh were proposed as injection sites. The exposure after injection into thigh was not different from that after in abdomen. However, pramlintide exposure as AUC was 20-36% higher after arm injection compared to that in abdomen for obese patients. Therefore, arm should be cautiously considered as an alternative injection site for obese patients because of hypoglycemic safety concern with the higher exposure.
- The primary pharmacodynamic endpoint was the plasma glucose concentration-time curve from time zero to last time point ($AUC_{0\text{-}4hr}$). However, the AUC was not an optimal PD endpoint because of averaging nature between below and above the baseline (fasting glucose level).
- Inhibition of postprandial glucose excursion was one of the proposed beneficial effects of pramlintide. Mean maximum postprandial glucose elevation ($C_{max,glu}$) was observed as 87.2mg/dL for placebo (insulin alone), 77.7mg/dL for pramlintide dosing 15 minutes before breakfast, and 47.2mg/dL for pramlintide dosing immediate before breakfast in type 1 using lispro insulin group. However, the elevated glucose levels ($C_{max,glu}$) were observed at the last sampling of the study (i.e., 4 hours after breakfast). The some levels were as close as the maximum postprandial glucose elevations of placebo. In these regards, it was premature to assess the beneficial role of pramlintide to postprandial glucose excursion based on the results in the type 1 using lispro insluin. Comparison of individual data across treatments may provide further insight of the results that were with significant inter-subject variability.

5 Labeling Comments

The labeling comments will be available when the final decision on the approvability on the application is made.

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/s/

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Division of Pharmaceutical Evaluation-II
Office of Clinical Pharmacology and Biopharmaceutics

NDA:	21-332	**Relevant IND:**	39,897
Brand Name:	Symlin™	**Generic Name:**	Pramlintide Acetate
Concentrations:	Vial – 0.6 mcg/mL sterile injection in 5 mL vials [ ]		
Sponsor:	Amylin Pharmaceuticals, Inc. 9373 Towne Centre Drive, San Diego, CA 92121		
Submission Date:	7-DEC-2000; 5-APR-2001		
Advisory Committee:	26-JUL-2001		
CPB Reviewer:	Steven B. Johnson, Pharm.D.		
CPB Team Leader:	Hae-Young Ahn, Ph.D.		
Acknowledgements:	Daniel Davis, M.D.; S.W. Johnny Lau, Ph.D.; Todd Sahlroot, Ph.D.		

EXECUTIVE SUMMARY

On December 7, 2000, Amylin Pharmaceuticals submitted NDA 21-332 in support of Symlin™ (pramlintide acetate) injection. Pramlintide is the synthetic analogue of the 37-amino acid polypetide, amylin. The proposed mechanism of pramlintide action is complex, with regulation of postprandial glucagon concentrations and altered gastric emptying rate being the most well described. —— formulation—of Symlin™ have been proposed for marketing, a 0.6 mg/mL (vial) formulation that will be administered by syringe [ ] Symlin™ has been proposed for use as adjunctive therapy to insulin in patients with type 1 or insulin-requiring type 2 diabetes mellitus (DM).

Included in this application were 28 clinical pharmacology and biopharmaceutics related studies or reports. Of these studies, 19 were used to make the CPB recommendation. Many of the early studies were found to be unacceptable for review due to formulation and/or assay issues. Common to the studies that were utilized in this review included: a formulation pH of 4.0 and/or the use of the current immunoenzymetric assay (IEMA) for pramlintide pharmacokinetic (PK) studies.

The following is a brief description of Symlin™ attributes. First of all, there is a high degree of inter-subject variability for all PK parameters, except $t_{½}$ and T_{max}. This drug is absorption rate limited, has a time to maximum pramlintide plasma concentration (T_{max}) of approximately 20 minutes, and a half-life of about 50 minutes. Pramlintide is metabolized to des-lysine pramlintide, which has 100% of the activity as pramlintide, and other none reactive fragments. There is no apparent drug accumulation following multiple doses in either type 1 or type 2 diabetes patients.

In order to take full advantage of pramlintide's delayed gastric emptying effect, Symlin™ should be administered about 15 minutes before a meal – this timing would correspond with the pramlintide T_{max}. Symlin™ should be administered subcutaneously into the tissue of the anterior abdominal wall only, with a maximum dose of 360 mcg/day – divided BID, TID, or QID.

During the course of this review, a series of questions were generated to address pertinent issues that were thought to be key for the approval of this application. The most prominent of these questions are:

1) **Is the analytical method used to detect pramlintide in human plasma precise and accurate?**
   Yes, the immunoenzymetric assay used to detect human plasma pramlintide exhibits precision and accuracy estimates that are acceptable. However, it should be noted that the samples used in the quality control analysis were sufficiently far enough away from the calibration limits and the lower limit of quantitation as to create some concern about the plasma concentrations that fall between the LLOQ and the lowest quality control sample.

2) **Is there any assay interference from endogenous substances or metabolites?**
Yes, this assay is susceptible to interference by endogenous amylin, the des-lysine pramlintide metabolite, and human anti-mouse antibody (HAMA). Also, the values reported as pramlintide concentrations are actually pramlintide, the pramlintide metabolite, and amylin.

3) **What is the absolute bioavailability of Symlin™?**
The bioavailability of a subcutaneously administered dose of Symlin™, relative to an equivalent intravenously administered dose of Symlin™, is approximately 37%. Pramlintide exhibits absorption rate limited pharmacokinetics.

4) **What effect does pH, mixing, volume, or concentration have on the bioavailability of Symlin?**
The formulation pH has a significant effect on the bioavailability of Symlin™. A formulation pH of 4.7 was shown to exhibit a 25% reduction in bioavailability compared to the to-be-marketed pH 4.0 formulation.

Compatibility studies, that would describe what substances could be mixed with pramlintide, were not performed. However, in interaction studies where pramlintide and insulins were mixed in the same syringe, pramlintide and sometimes insulin pharmacokinetics were significantly altered.

Volume and concentration had no apparent effect on Symlin™ bioavailability.

5) **Does Symlin™ exhibit dose proportionality over the entire proposed dosing range, 30 mcg to 180 mcg?**
No, Symlin™ does not exhibit dose proportionality over the entire proposed dosing range. However, studies in healthy volunteers and patients with type 1 and type 2 diabetes demonstrate a dose-related exposure. Apparent clearance in normal healthy subjects ranged from 2.36 L/min to 2.87 L/min for the dosing range of 30 mcg to 120 mcg.

1) **Given that Symlin™ will be available in — concentrations, are these formulations bioequivalent?**
Bioequivalence was established [ ] the vial formulation administered with a syringe. Ninety-five percent confidence limits were within the 80% to 125% boundaries for — C_{max} and AUC.

7) **Since there will be three suppliers of pramlintide material for Symlin™, are there any PK-related concerns about using multiple sources of this protein?**
No, there are no outstanding concerns related to the multiple sources of pramlintide. Information submitted in the form of a BE study was sufficient to conclude that pramlintide material from [ ] were equivalent. Material from both [ ] was used throughout the development program and was not considered different.

8) **Are there any differences between the pramlintide PK profiles of type 1 and type 2 diabetes patients?**
Yes, there is a difference between the pramlintide PK profiles of type 1 and type 2 diabetes patients. In the studies evaluated in this application, patients with type 2 diabetes exhibited lower relative pramlintide plasma concentrations than did patients with type 1 diabetes. This fact is reflected in the proposed diabetes type-specific dosing regimens.

9) **Pramlintide is renally eliminated, what effect does renal insufficiency have on pramlintide PK?**
The exact effect that renal insufficiency has on pramlintide PK cannot be stated with absolute certainty due to the design limitations of the renal study (e.g., parallel design with 3 to 8 subjects per group and inherent inter-subject variability). However, there was no observed trend in the data to suggest that renal insufficiency adversely effects pramlintide PK. It should be kept in mind that pramlintide dosing in humans has spanned 30 mcg to 10 mg in single dose studies.

10) **Can pramlintide be mixed with insulin(s)?**
No, pramlintide should not be mixed in the same syringe with any insulin. The sponsor evaluated mixing effects of pramlintide with rapid, short, intermediate, and long-acting insulins. The results

suggest that pramlintide PK, and sometimes insulin PK, is compromised when these agents are mixed.

1) **Since pramlintide delays gastric emptying time, what effect does Symlin™ have on orally administered medications?**
Two studies evaluated the effect Symlin™ has on the PK of Lo/Ovral, an oral contraceptive, and ampicillin, a relatively acid-stable antibiotic. Results indicate that norgestrel, the progestin component of Lo/Ovral, achieves significantly lower and delayed C_{max} values when administered 15 minutes after a dose of Symlin™. However, there was no difference in the extent of norgestrel exposure.

Conversely, pramlintide appeared to have no effect on ampicillin AUC or C_{max}. However, T_{max} was increased by approximately one hour.

The conclusion drawn from these studies would suggest that orally administered drugs that are expected to have a rapid onset of action, or those that are adversely affected (e.g., degraded) by prolonged gastric retention times, should be administered at least one hour prior to dosing pramlintide.

12) **What effect does a morning dose of pramlintide have on the gastric emptying of a lunchtime meal?**
There was no observed interference of a morning dose of pramlintide on a lunchtime meal eaten approximately 4 hours after pramlintide administration. This is consistent with pramlintide's attributes of a relatively short half-life of pramlintide, ~ 50 minutes, and the fact that there is no indication of pramlintide accumulation.

Based upon the results of the studies that answer the above questions, it has been concluded that Amylin, Inc. has supplied sufficient information to the PK section for Symlin™, NDA 21-332, to have been adequately evaluated.

RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics has reviewed NDA 21-292 for Symlin™ (pramlintide acetate) and finds the application acceptable, pending the indicated labeling changes (see ***Comments to Sponsor*** and ***Labeling Changes***).

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APPENDIX INDEX

BACKGROUND – (from sponsor)

In people without diabetes, plasma glucose concentrations are tightly regulated by the co-secretion of the hormones amylin and insulin from the pancreatic β-cells in response to nutrient intake, and by the glucagon secretion from pancreatic α-cells in response to a variety of stimuli, including hypoglycemia and elevated concentrations of amino acids. In people with type 1 diabetes, the pancreatic β-cells are usually destroyed by an autoimmune process, leaving patients deficient in both insulin and amylin. In people with type 2 diabetes, insulin resistance leads to an increased demand for insulin, and initially results in increases in β-cell secretion of both insulin and amylin. Over time, β-cell secretion fails, and relative deficiencies of both insulin and amylin occur in conjunction with inappropriate fluctuations in glucose concentrations, overt hyperglycemia, and increased risk of hypoglycemia.

Results of nonclinical and clinical studies indicate that the 37-amino acid polypeptide amylin and the amylin analogue pramlintide contribute to glucose regulation through several mechanisms including reducing the postpranidal rise in glucagon concentrations without impeding the glucagon response to insulin-induced hypoglycemia, and regulating the rate of nutrient delivery to the small intestine via an effect on gastric emptying. It has been proposed that amylin's effect on gastric emptying may be exerted via a central mechanism involving specific binding sites in the area postrema of the brain, with outflow through the efferent pathways of the vagus nerve to the gastrointestinal system, rather than by direct action on the stomach. Elevated glucagon concentrations favor increased rates of hepatic glucose release, and a reduction in postprandial glucagon concentrations should result in lower rates of hepatic glucose output during the postprandial period, thus favoring lower postprandial glucose concentrations. It has been demonstrated that hypoglycemia overrides these effects.

Immunoassay of amylin in healthy human volunteers indicates fasting concentrations between 4 and 8 pmol/L, increasing two- to three-fold following ingestion of a mixed meal or an oral glucose load. In patients with type 1 diabetes mellitus, amylin concentrations are near or below the limit of quantitation under fasting conditions and do not increase in response to nutrient stimuli. In patients with type 2 diabetes mellitus or with impaired glucose tolerance, fasting amylin concentrations are comparable to those seen in healthy human subjects. However, the postprandial responses vary considerably. The postprandial responses tend to be decreased in relation to the prevailing level of glycemia and are virtually absent in patients with type 2 diabetes who have progressed to insulin therapy. Amylin deficiency in patients with diabetes mellitus may contribute to impaired glucoregulation.

TERMS and ABBREVIATIONS

AA	Amino acid(s)
Agency	Food and Drug Administration
AUC	Area under the plasma-concentration-time curve
BA	Bioavailability
BE	Bioequivalence
BMI	Body Mass Index
C_{max}	Maximum drug concentration
DMEDP	Division of Metabolic and Endocrine Drug Products
OCPB	Office of Clinical Pharmacology and Biopharmaceutics
NDA	New Drug Application
T_{max}	Time of maximum drug concentration (C_{max})
$t_{1/2}$	Drug elimination half-life

DRUG CHARACTERISTICS

Drug Chemistry

Pramlintide acetate is a synthetic analogue of the endogenous human polypeptide, amylin. Pramlintide differs from amylin in its replacement of amino-acid (AA) residues at 25 (alanine), 28 (serine), and 29 (serine) of the 37-AA amylin peptide, with proline residues.

Pramlintide acetate is an odorless white powder, is soluble in water, has a molecular weight of 3949.9, and a molecular formula of $C_{171}H_{267}N_{51}O_{53}S_2{}^*xC_2H_4O_2$, where x is variable. The structural formula is shown below, and includes the disulfide bridge between the two cysteine residues:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-
Asn-Asn-Phe-Gly-**Pro**-Ile-Leu-**Pro**-**Pro**-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH_2 acetate

Drug Formulation

Multiple formulations (i.e., vial = 26; [ ] have been described in the development of Symlin™. Of these formulations, AC-0137-F22 (vial) [ ] were chosen for marketing. The formulations for dosage units are presented in **TABLE 1**:

TABLE 1: Symlin™ Formulations

	Vial – F22
Strength	0.6 mcg/mL (5 mL)
pH	4.0
Pramlintide	0.60 g/L
Acetate	
Mannitol	
Metacresol	
Acetic Acid	
Sodium Acetate Trihydrate	
Water for Injection	

*Meets both the European Pharmacopeia (EP) and United States Pharmacopeia (USP) monographs

Early pharmacokinetic studies utilized formulations other than those shown above that differed mainly in their relative pH values – those studies were not reviewed. The data that provided the basis for the Clinical Pharmacology and Biopharmaceutics recommendation was generated using the above to-be-marketed formulations.

Additionally, the sponsor is using three suppliers of pramlintide in the manufacture of Symlin™, [ ] (see **HUMAN PK – BIOEQUIVALENCE**).

ANALYTICAL

Is the analytical method used to detect pramlintide in human plasma precise and accurate?

Is there any assay interference from endogenous substances or metabolites?

The detection of pramlintide is dependent upon a validated immunoenzymetric assay (IEMA) method that relies on two monoclonal mouse antibodies (Ab): a capture Ab (F024-4.4) and a detection Ab (F025-27.4). Neither Ab is specific for amylin or pramlintide. Antibody F024-4.4 binds near the amino terminus of pramlintide and Ab F025-27.4 binds to the amidated carboxy terminus of pramlintide. The F025-27.4 Ab is conjugated to alkaline phosphatase and a fluorescent substrate, 4-methylumbelliferyl phosphate (4-MUP). This method is able to detect bound, conjugated antibody, using a microplate fluorometer. Relative fluorescence units (RFU) are correlated with concentration using a calibration curve defined in the same assay. This assay uses [ ]

In **TABLE 2**, the quality control samples used in the assays for studies 137-142 and 137-145 are presented. These two studies were chosen because they were the two pivotal bioequivalence studies submitted with this application. Both accuracy and precision were within accepted parameters for these studies. However, there is a concern related to the quality control samples.

Quality control samples were measured at the sponsor defined values of low, middle, and high. However, because the extreme values were far away from the limits of the calibration curve, there exists some doubt about the values obtained at the extremes, especially those plasma pramlintide values that fall between the LLOQ and the lowest quality control sample value. Therefore, because of this issue and the cross-reactivity with endogenous amylin, pharmacokinetic analysis will be confined to C_{max} and AUC_{0-t}.

Assay interference was documented for endogenous amylin and for des-lysine pramlintide with 35% and 100% cross-reactivity, respectively. This cross-reactivity results in a total pramlintide concentration that is actually a combination of amylin, des-lysine pramlintide, and pramlintide. Since under fasting conditions endogenous amylin concentrations are usually undetectable, and the fact that the des-lysine pramlintide

metabolite is equipotent with pramlintide, these findings are not likely to undermine the value of the assay.

TABLE 2 – Assay Quality Control Results from Two Pivotal Bioequivalence Studies

Study	137-142	137-145
LLOQ (pmol/L)		
Calibration Range		
Precision (%CV)		
Accuracy (%)		
Stability	freeze/thaw cycles	
Storage	1 year @ either –20°C or –70°C	
Crossreactivity	35% – amylin; 100% – des-lysine pramlintide	
Homology	92% – amylin; 97% – des-lysine pramlintide	

One important point. This IEMA utilizes monoclonal antibodies from a murine source – and, many humans carry the human anti-mouse antibody (HAMA). HAMA, present in plasma, binds to the mouse antibodies in the assay, which can result in a false signal, either positive or negative. Strategies for reducing this interference include: immunosuppressant therapy, and the use of humanized, polyethylene glycolated, or Fab fragments of antibody agents. The sponsor chose to reduce the HAMA interference by using a diluent that contained a high concentration of non-specific mouse IgG which competes with the specific mouse monoclonal antibodies for the human anti-mouse antibody binding sites.

The above point was raised because several subjects were determined to be unevaluble in several PK studies due to this assay interference. The Agency strongly encourages the sponsor to develop an assay that does not utilize murine antibodies.

HUMAN PK – BIOAVAILABILITY/BIOEQUIVALENCE

Symlin is a parenterally (i.e., subcutaneously) administered sterile solution. As such, knowing the absolute bioavailability is crucial. It is also imperative that there is a good understanding of the effect that physiochemical alterations have on the product's bioavailability. Therefore, there are two primary questions that should be asked:

What is the absolute bioavailability of Symlin™?

What effect does pH, mixing, volume, or concentration have on the bioavailability of Symlin?

Absolute Bioavailability

The absolute bioavailability for Symlin was determined in an open-label, randomized, four-period crossover study (137-125) in 40 (39 completed[i]) healthy male subjects between 18 and 41 years of age. The four treatments consisted of: A) 60 mcg (0.1 mL) pH 4.7 pramlintide administered subcutaneously (formulation F11); B) 60 mcg (0.1 mL) pH 4.0 pramlintide administered subcutaneously (formulation F22); C) 60 mcg (0.2 mL) pH 4.0 pramlintide administered subcutaneously (formulation F21); and D) 60 mcg (0.1 mL) pH 4.0 pramlintide administered intravenously (formulation F22). Each of the treatments was separated by a 1-week washout period.

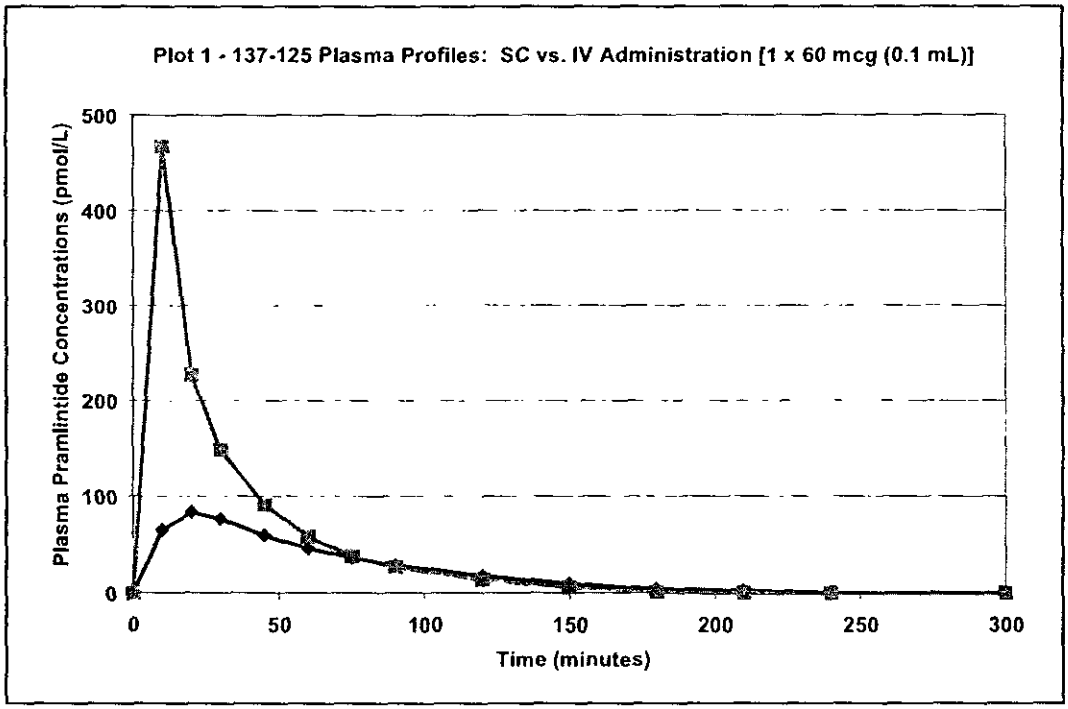
Results, presented in **TABLE 3** and **PLOT 1**, show that the absolute bioavailability (f) of a 60 mcg dose of pramlintide, in a volume of 0.1 mL, administered to healthy volunteers under fasting conditions, is 37%.

However, because the sampling times for the IV administered pramlintide did not include a sufficient number of early time points, the absolute bioavailability of pramlintide may be overestimated.

TABLE 3 – Pramlintide Plasma Concentrations: SC vs. IV Administration

Parameter	Units	Tx B: 60 mcg (0.1 mL) SC pH 4.0			Tx D: 60 mcg (0.1 mL) IV pH 4.0		
		Mean	SD	%CV	Mean	SD	%CV
C_{max}	pmol/L	89.23	24.01	26.9	--	--	--
T_{max}	minutes	20.5	6.51	31.9	--	--	--
AUC_{0-t}	pmol*min/L	6234	2154	34.6	17,950	6834	38.1
AUC_{0-inf}	pmol*min/L	6803	2253	33.1	18,420	6795	36.9
$t_{1/2}$	minutes	43.6	13.6	31.2	33.4	7.94	23.8
C_{max} / BMI	pmol/L	3.90	1.19	30.5	--	--	--
AUC_{0-t} / BMI	pmol*min/L	272.0	99.39	36.5	789.4	325.4	41.2
AUC_{0-inf} / BMI	pmol*min/L	297.2	103.6	34.9	809.6	324.4	40.1

Analysis was also conducted to determine if body mass index (BMI) had any impact on the coefficient of variation for both C_{max} and AUC. Results do not indicate a significant difference between the uncorrected parameters and those corrected for BMI. This suggests that BMI has little influence on the pharmacokinetics of pramlintide in healthy males.



Effect of pH on Bioavailability

Formulation pH was shown to have a significant effect on the bioavailability of subcutaneously administered pramlintide. In study 137-125, a pH 4.7 formulation (F11) was compared with the to-be-marketed pH 4.0 formulation (F22). Results, as shown in **TABLE 4**, revealed that the pH 4.7 formulation had a 25% reduction in AUC and a 35% decrease in the mean C_{max}, when compared with the pH 4.0 formulation. Because of this pH issue, earlier studies which utilized the pH 4.7 formulation were not considered in this review.

TABLE 4 – Pramlintide Plasma Concentrations: pH 4.7 vs. pH 4.0

Parameter	Units	Tx A: 60 mcg (0.1 mL) SC pH 4.7			Tx B: 60 mcg (0.1 mL) SC pH 4.0		
		Mean	SD	%CV	Mean	SD	%CV
C_{max}	pmol/L	59.70	24.01	40.2	89.23	24.01	26.9
T_{max}	minutes	20.7	13.2	63.8	20.5	6.51	31.9
AUC_{0-t}	pmol*min/L	4485	2206	49.2	6234	2154	34.6
AUC_{0-inf}	pmol*min/L	5193	2208	42.5	6803	2253	33.1
$t_{1/2}$	minutes	49.3	18.9	38.3	43.6	13.6	31.2
C_{max} / BMI	pmol/L	2.62	1.15	43.9	3.90	1.19	30.5
AUC_{0-t} / BMI	pmol*min/L	195.6	100.3	51.3	272.0	99.39	36.5
AUC_{0-inf} / BMI	pmol*min/L	226.4	99.89	44.1	297.2	103.6	34.9

Effect of Volume on Bioavailability

The effect of the volume of SC injections on pramlintide PK was described in several studies, with the most indicative examples being the single/multiple dose studies conducted in type 1 and type 2 diabetes patients. In study 137-143, patients with type 1 DM were administered either a 0.03 mL, 0.06 mL, or 0.09 mL of the 1.0 mg/mL cartridge formulation. In study 137-144, patients with type 2 DM were administered either a 0.06 mL, 0.09 mL, 0.12 mL, or 0.18 mL of the 1.0 mg/mL cartridge formulation. In both cases, $AUC_{0-300\ min}$ and C_{max} values exhibited expected dose related changes (**see HUMAN PK – TARGET POPULATION**). Similar results were observed in the absolute BA study, 137-125, where a 0.1 mL dose of the 0.6 mg/mL vial formulation was compared with 0.2 mL of a 0.3 mg/mL "test" formulation. These combined results suggest that the volume of the injection has no significant impact on the bioavailability of Symlin.

Effect of Mixing on Bioavailability

The idea of mixing is an important consideration with Symlin, as it will be used as an adjunct to insulin therapy. With this in mind, the sponsor has conducted numerous studies in which Symlin was mixed in the same syringe with different insulin products (e.g., regular insulin, insulin lispro, NPH insulin, etc.). The overwhelming conclusion from reviewing these studies is that Symlin should not be mixed in the same syringe with insulin because Symlin PK is significantly affected and efficacy will likely be compromised (see **HUMAN PK – EXTRINSIC – Drug-Drug Interactions**).

Also, due to the importance of pH on the bioavailability of Symlin, mixing Symlin in the same syringe with any agent that can potentially alter the pH is not recommended.

Effect of Concentration on Bioavailability

The effect of concentration on bioavailability was evaluated in two studies, 137-125 and 137-142. Study 137-125 compared a single 60 mcg dose at a concentration of 0.1 mL at pH 4.0 (F22 – to be marketed formulation – reference) with a single 60 mcg dose at a concentration of 0.2 mL at pH 4.0 (F21 – test). Results indicate that a doubling of the injection volume, and hence a halving of the concentration, results in a 6.5% reduction in the relative BA (AUC_{0-t}).

Study 137-142, discussed in HUMAN PK – Bioequivalence, demonstrated that a 1.0 mg/mL formulation (cartridge) was bioequivalent to a 0.6 mg/mL formulation (vial). It also showed that the relative bioavailability of the cartridge formulation to the vial formulation was about 97% ($AUC_{0-300\ min}$).

Therefore, small changes in drug concentration do not appear to have a significant impact on the bioavailability of Symlin.

Dose Proportionality

Does Symlin™ exhibit dose proportionality over the entire proposed dosing range, 30 mcg to 180 mcg?

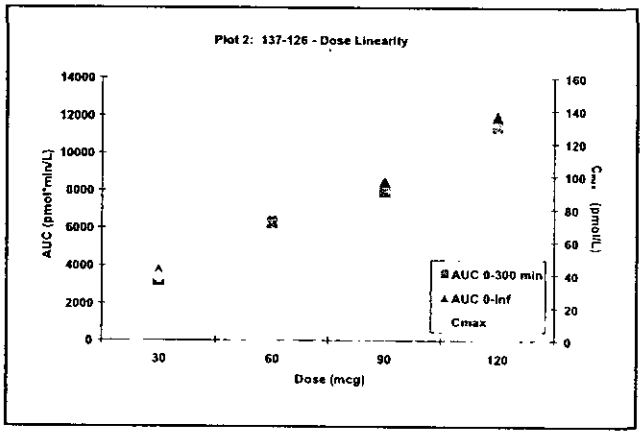
In order to characterize dose proportionality, the sponsor has conducted a randomized four-way crossover study in 40 (38 completed) healthy male subjects using four single subcutaneous doses of pramlintide administered at constant volume (0.2 mL). The treatments were as follows: Tx A – 30 mcg

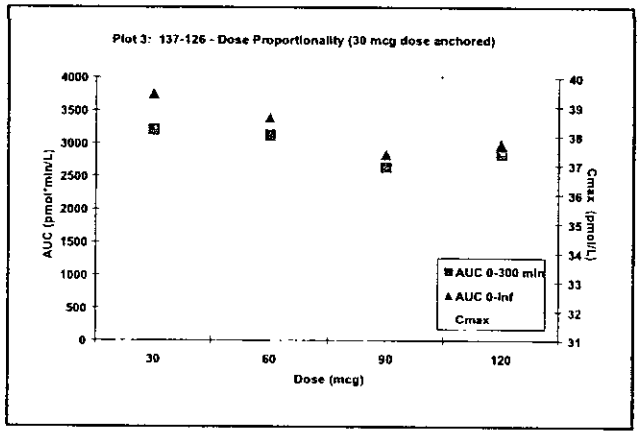
(as 0.1 mL of formulation F21 (lot 96-0201GB) plus 0.1 mL placebo (lot 95-0504GE); Tx B – 60 mcg (as 0.1 mL of formulation F22 (lot 96-0503JB) plus 0.1 mL placebo (lot 95-05804GE); Tx C – 90 mcg (as 0.1 mL of formulation F24 (lot 96-0506JB) plus 0.1 mL placebo (lot 95-0504GE); and Tx D – 120 mcg (as 0.2 mL of formulation F22(lot 96-0503JB). Each treatment phase was separated by a 1-week washout period.

Results (**TABLE 5**) indicate that SC administered pramlintide exhibits near linear kinetics between 30 and 120 mcg in normal healthy subjects – with a definite dose related increase in the PK parameters (see **Plots 2 & 3**). However, this study failed to demonstrate dose proportionality.

TABLE 5 – Pramlintide PK Profiles in Normal Healthy Subjects – Single Dose

Parameter	Units	30 mcg	60 mcg	90 mcg	120 mcg
C_{max}	pmol/L	39.26 ± 9.21	79.44 ± 20.50	102.48 ± 30.23	146.99 ± 35.50
AUC_{0-300}	pmol*min/L	3215 ± 1122	6261 ± 2401	7939 ± 2848	11380 ± 3839
T_{max}	min	21.4 ± 8.79	19.5 ± 7.69	19.1 ± 7.70	21.3 ± 8.36
$t_{½}$	min	54.9 ± 14.5	49.2 ± 15.3	51.1 ± 20.0	48.1 ± 12.8
Cl (apparent)	L/min	2.36	2.42	2.87	2.67
Mean ± SD					





Two additional studies, 137-143 and 137-144, considered dose linearity and dose proportionality in type 1 and type 2 diabetes patients, respectively. Results were similar to those seen in study 137-126, with linearity being demonstrated between the range of 30 and 180 mcg (see **HUMAN PK – TARGET POPULATION**).

HUMAN PK – BIOEQUIVALENCE

Given that Symlin™ will be available in — oncentrations, are these formulations bioequivalent?

Cartridge vs. Vial

— dosage forms have been [ ] by the sponsor, [ ] and a vial dosage form, to be used for syringe administration. Study 137-142 was conducted to establish bioequivalence between these two dosage forms. In this single center, open-label, randomized, two-period crossover study consisting of two evaluation periods with a 24-hour washout period between dosing events, a single 60 mcg dose administered by pen from the cartridge form (1.0 mg/mL) was compared with a single 60 mcg dose administered by syringe from the vial form (0.6 mg/mL) in 30 subjects (20 females and 10 males). All doses were administered into the subcutaneous tissue of the anterior abdominal wall.

TABLE 6 – Pramlintide PK Parameters: Cartridge Formulation vs. Vial Formulation

Parameter	Units	Tx A: Pen System 60 mcg @ 1.0 mg/mL			Tx B: Vial (syringe) 60 mcg @ 0.6 mg/mL		
		Mean	SD	%CV	Mean	SD	%CV
C_{max}	pmol/L	71.2	23.75	33.4	65.7	21.89	33.3
T_{max}	minutes	21.8	9.51	43.6	25.2	15.17	60.2
$AUC_{0\text{-}300\ min}$	pmol*min/L	5742.0	3366.3	58.6	5695.5	3489.35	61.3
$AUC_{0\text{-}inf}$	pmol*min/L	6532.8	3499.7	53.6	6704.2	3725.05	55.6
$t_{1/2}$	minutes	52.8	16.51	31.3	59.4	26.55	44.7

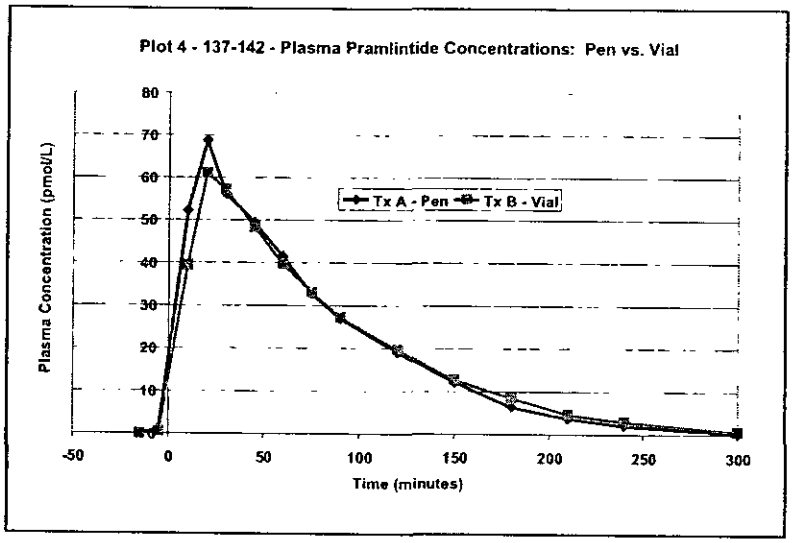
TABLE 7 – Pramlintide BE Comparison: Cartridge Formulation vs. Vial Formulation

Parameter	Units	Tx A: Pen System	Tx B: Vial (syringe)	Tx: A/B	90% CI[d]		p-value[e]
		Mean[b]	Mean[b]	Ratio[c]	Low	High	
C_{max}[a]	pmol/L	67.3	62.1	108.4	100.0	117.4	0.0986
T_{max}	minutes	20.0	20.0				0.1226[f]
$AUC_{0\text{-}300\ min}$[a]	pmol*min/L	4845	4679	103.6	93.3	115.0	0.5742
$AUC_{0\text{-}inf}$[a]	pmol*min/L	5673	5673	100.0	90.5	110.5	0.9993
$t_{1/2}$	minutes	52.8	59.4				0.1554

a – Parameters were natural log-transformed before analysis.
b – Means for the test (pen) and reference (syringe) treatment formulations;
- (geometric means – anti-log of the means of the logs – for natural log-transformed parameters); and
- (arithmetic means are presented for $t_{1/2}$ and median values presented for T_{max}).

c – Ratio of geometric means calculated as Test/Reference.
d – 90% CI of the geometric means ratio T/R.
e – P-value from ANOVA (sequence, subject-within-sequence, period, & treatment) for testing treatment differences.
f – P-value from the Wilcoxon signed-rank test for the difference of 2 treatment formulations.

Results of this study indicate that the cartridge and vial formulations are bioequivalent when 60 mcg is administered subcutaneously to healthy individuals. This study also suggests that small differences in concentration, i.e., 1.0 mg/mL (cartridge) vs. 0.6 mg/mL (vial), results in non-significant differences in the rate of absorption and no detectable difference in the extent of absorption for this product (see **PLOT 4**).



Since there will be three suppliers of pramlintide material for Symlin™, are there any PK-related concerns about using multiple sources of this protein?

[ ] **Material**

There has been a great deal of concern about the adequacy of the chemical characterization of peptides and their associated process impurities. Since the sponsor is proposing that three independent suppliers of pramlintide provide material for Symlin, the Agency has requested comparative bioavailability information. Specifically, a comparison was requested between [ ]; material, which has never been used in clinical studies, and either [ ] material, both of which have been used extensively in clinical development and are thought to be equivalent.

As such, the sponsor has submitted a two-way crossover design study (137-145) conducted in 30 normal healthy male and female subjects. Treatments consisted of a single 60 mcg dose from a 1.0 mg/mL formulation in cartridge form with active ingredient manufactured by [ ] [AC137-F28 (99-0603KB) that was compared with a single 60 mcg dose from a 1.0 mg/mL formulation in cartridge form with active ingredient manufactured by — [AC137-F28 (99-0602KB)].

Results of this study (see **TABLE 8**) clearly show that the F28 cartridge formulation produced from the [ ] pramlintide material is bioequivalent to the F28 cartridge formulation produced from the — material.

TABLE 8 – Pramlintide BE Comparison: [ ] vs. [ ] Pramlintide Material

Parameter	Units	Tx A: — Mean[b]	Tx B: — Mean[b]	Tx: A/B Ratio[c]	90% CI[d] Low	90% CI[d] High	p-value[e]
C_{max}[a]	pmol/L	50.4	51.8	0.97	89.4	105.9	0.5817
T_{max}	minutes	17.8	16.2				0.4747
$AUC_{0\text{-}300\ min}$[a]	pmol*min/L	2463.1	2554.4	0.96	85.6	108.6	0.6067
$AUC_{0\text{-}inf}$[a]	pmol*min/L	3631.6	3609.9	1.01	89.6	113.1	0.9279
$t_{1/2}$	minutes	55.8	56.9				0.8657

a – Parameters were natural log-transformed before analysis.
b – Means for the test [ ] and reference [ ] treatment formulations;
- (geometric means – anti-log of the means of the logs – for natural log-transformed parameters); and
- (arithmetic means are presented for $t_{1/2}$ and T_{max}).

c – Ratio of geometric means calculated as Test/Reference.
d – 90% CI of the geometric means ratio T/R.
e – P-value from ANOVA (sequence, subject-within-sequence, period, & treatment) for testing treatment differences.

HUMAN PK – TARGET POPULATION

Are there any differences between the pramlintide PK profiles of type 1 and type 2 diabetes patients?

Single and multiple dose PK/PD studies were conducted in both type 1 and type 2 diabetes patients. Common findings to both of these studies were: dose linearity over the study-specific dosage ranges, similar confounding factors that led to inconclusive pharmacodynamic conclusions (e.g., incomplete insulin usage records), and no apparent dose accumulation between treatments days 1 and 5. Of note, is the observation that concentrations in type 2 patients tend to be lower than those seen in type 1 patients.

Study 137-143 assessed the single and multiple dose PK profiles of SC administered pramlintide in 11 type 1 diabetes patients. This study was a randomized, three-treatment, three-way crossover design with two dosing frequency groups: three times daily (TID) and four times daily (QID). The group 1 treatments consisted of either 30 mcg, 60 mcg, or 90 mcg pramlintide doses administered SC using the 1.0 mg/mL cartridge formulation and given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses). The group 2 treatments were similar to the group 1 treatments, but with the addition of a fourth dose administered just prior to an evening snack (17 consecutive doses). Results are presented in **TABLE 9**.

TABLE 9 – Pramlintide PK Profiles in Type 1 Diabetes Patients – Single & Multiple Dosing

Parameter	Day	30 mcg TID	60 mcg TID	90 mcg TID	30 mcg QID	60 mcg QID	90 mcg QID
C_{max} (pmol/L)	1	41.9 ± 22.9	64.5 ± 23.7	99.4 ± 31.4	36.5 ± 10.2	66.9 ± 25.0	123.9 ± 40.5
	5	37.6 ± 22.8	74.4 ± 20.0	92.7 ± 26.7	40.7 ± 20.0	70.7 ± 23.3	98.6 ± 30.1
T_{max} (hr)	1	0.273 ± 0.0753	0.365 ± 0.237	0.321 ± 0.115	0.274 ± 0.0751	0.276 ± 0.0794	0.273 ± 0.072
	5	0.328 ± 0.120	0.334 ± 0.113	0.288 ± 0.083	0.249 ± 0.002	0.278 ± 0.080	0.275 ± 0.082
AUC_{0-t} (pmol*hr/L)	1	32.86 ± 28.40	74.14 ± 34.26	118.5 ± 65.37	20.10 ± 11.46	51.67 ± 33.37	115.9 ± 54.29
	5	26.45 ± 21.93	79.68 ± 48.98	104.8 ± 63.53	24.20 ± 18.76	63.88 ± 49.83	102.0 ± 41.77
AUC_{0-inf} (pmol*hr/L)	1	81.37 ± 22.17	102.6 ± 37.4	144.2 ± 65.74	48.88 ± ?	72.01 ± 37.82	129.1 ± 55.74
	5	64.96 ± 9.63	117.2 ± 44.39	136.3 ± 66.70	66.43 ± 15.99	98.34 ± 56.22	124.1 ± 46.12
$t_{1/2}$ (hr)	1	1.12 ± 1.01	0.970 ± 0.337	0.774 ± 0.281	0.595 ± ?	0.656 ± 0.169	0.645 ± 0.165
	5	0.726 ± 0.239	0.789 ± 0.326	0.722 ± 0.230	0.713 ± 0.173	0.724 ± 0.303	1.02 ± 1.02
k_{el} (1/hr)	1	0.887 ± 0.374	0.796 ± 0.278	1.03 ± 0.423	1.16 ± ?	1.13 ± 0.316	1.14 ± 0.284
	5	1.02 ± 0.282	1.00 ± 0.384	1.03 ± 0.281	1.00 ± 0.243	1.07 ± 0.342	1.03 ± 0.496
Mean ± SD							

Study 137-144 assessed the single and multiple dose PK profiles of SC administered pramlintide in 12 type 2 diabetes patients. This study was a randomized, three-treatment, three-way crossover design with two dosing frequency groups: two times daily (BID) and three times daily (TID). The group 1 treatments consisted of either 60 mcg, 120 mcg, or 180 mcg pramlintide doses administered SC using the 1.0 mg/mL cartridge formulation (lot # 97-0403KB) and given just prior to breakfast and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (9 consecutive doses). The group 2 treatments included 60 mcg, 90 mcg, and 120 mcg doses of pramlintide administered just prior to breakfast, lunch, and dinner (13 consecutive doses). Results are presented in **TABLE 10**.

TABLE 10 – Pramlintide PK Profiles in Type 2 Diabetes Patients – Single & Multiple Dosing

Parameter	Day	60 mcg BID	120 mcg BID	180 mcg BID	60 mcg TID	90 mcg TID	120 mcg TID
C_{max} (pmol/L)	1	50.8 ± 20.3	117.4 ± 116.4	151.3 ± 67.2	36.4 ± 18.5	55.7 ± 25.7	74.0 ± 24.6
	5	55.9 ± 24.6	97.8 ± 34.0	137.3 ± 36.4	42.2 ± 20.2	60.1 ± 24.4	77.2 ± 28.2
T_{max} (hr)	1	0.331 ± 0.107	0.341 ± 0.105	0.368 ± 0.221	0.290 ± 0.072	0.253 ± 0.008	0.260 ± 0.030
	5	0.284 ± 0.072	0.324 ± 0.124	0.339 ± 0.224	0.255 ± 0.009	0.301 ± 0.105	0.275 ± 0.071
AUC_{0-t} (pmol*hr/L)	1	62.39 ± 61.98	143.0 ± 139.9	179.4 ± 129.9	32.24 ± 46.23	53.93 ± 52.15	76.24 ± 54.37
	5	61.18 ± 52.32	123.1 ± 97.40	189.0 ± 147.8	35.06 ± 38.05	59.66 ± 46.52	91.19 ± 77.00
AUC_{0-inf} (pmol*hr/L)	1	119.1 ± 86.42	201.6 ± 160.0	201.0 ± 140.4	201.5 ± 111.6	124.9 ± 85.32	119.2 ± 79.45
	5	133.7 ± 67.68	163.4 ± 111.0	232.1 ± 166.2	107.7 ± 67.12	129.2 ± 63.29	152.4 ± 102.7
$t_{1/2}$ (hr)	1	1.24 ± 0.774	1.24 ± 0.832	0.926 ± 0.496	2.72 ± 1.49	1.38 ± 1.20	1.10 ± 0.778
	5	1.52 ± 1.09	1.02 ± 0.502	0.981 ± 0.406	1.42 ± 1.40	1.43 ± 0.991	1.15 ± 0.543
k_{el} (1/hr)	1	0.699 ± 0.301	0.760 ± 0.408	0.907 ± 0.385	0.300 ± 0.165	0.725 ± 0.338	0.811 ± 0.338
	5	0.594 ± 0.249	0.828 ± 0.369	0.814 ± 0.297	0.791 ± 0.425	0.615 ± 0.243	0.686 ± 0.231
Mean ± SD							

Comparison of tables 6 and 7 would suggest that, on average, patients with type 2 diabetes tend to exhibit lower total exposure than do patients with type 1 diabetes, when administered the same dose. It should also be noted that there is incredible variation in the PK parameters. This variability may be due to the route of administration, administration technique, and/or differences in body type.

Pramlintide is renally eliminated, what effect does renal insufficiency have on pramlintide PK?

Effect of Renal Insufficiency on Pramlintide PK

The sponsor conducted a study in order to evaluate the effect of renal insufficiency on pramlintide PK, based upon a rat nephrectomy model, a study that showed that basal levels of amylin were significantly higher in lean, non-diabetic patients with renal failure on chronic hemodialysis, and the fact that diabetes is a leading cause of renal disease.

Study 137-127 evaluated the PK of pramlintide in an open-label, parallel design study in 21 type 1 DM patients with varying degrees of renal function. Individuals were stratified by renal function into 1 of 4 categories based on creatinine clearance (CrCl): CrCl ≥ 90 mL/min; CrCl 60-89 mL/min; CrCl 30-59

mL/min; and CrCl < 30 mL/min. Each of the patients self-administered a single 60 mcg dose of pramlintide into the subcutaneous tissue of the anterior abdominal wall.

Based on the results of this study (**TABLE 11**), the sponsor concluded that "impaired renal function has no significant influence on pramlintide PK; therefore no dosing adjustment is required." However, it should be noted that because of the high inter-subject variability and the few number of patients enrolled in this parallel design study, there can be no definitive conclusion regarding dosing adjustment (i.e., this study is inconclusive). Therefore, dosing of patients with compromised renal function should be individualized based on efficacy and tolerability.

TABLE 11 – Renal Insufficiency – Plasma Pramlintide Parameters in Type 1 DM Patients

AUC_{0-Inf} (pmol*min/L)	Group I	Group II	Group III	Group IV
N	6	8	4	3
Mean ± SD	8396 ± 4360	5128 ± 2655	10538 ± 7064	5650 ± 4197
Min, max	[			]
C_{max} (pmol/L)				
Mean ± SD	84.17 ± 30.69	60.70 ± 30.58	86.10 ± 57.02	60.93 ± 35.00
Min, max	[			]
T_{max} (min)				
Mean ± SD	34.2 ± 23.06	21.9 ± 8.43	30.0 ± 20.41	15.0 ± 5.00
Min, max	[			]
$t_{½}$ (min)				
Mean ± SD	49.4 ± 10.85	64.3 ± 54.78	80.7 ± 21.94	49.4 ± 35.11
Min, max	[			]
Cl/F				
Mean ± SD	169.7 ± 147.9	236.1 ± 139.78	135.5 ± 111.65	438.2 ± 559.71
Min, max	[			]
Group I = CrCl ≥ 80 mL/min; Group II = CrCl 50-80 mL/min; Group III = CrCl 30-50 mL/min; Group IV = < 30 mL/min				

Drug-Drug Interactions

Symlin and Insulins

Can Symlin™ be mixed in the same syringe with insulin(s)?

The primary indication for Symlin™ is as an adjunct to insulin therapy. Since both pramlintide and insulin are administered via the subcutaneous route, for convenience (e.g., fewer daily injections), it would be ideal if the two could be mixed in the same syringe. With this in mind, the sponsor has conducted 4 studies (137-130, 137-115, 137-119, & 137-120) in which type 1 diabetes patients were administered pramlintide and insulin(s) at the same time but in different syringes, or pramlintide and insulin(s) in the same syringe. The insulins that were evaluated in these studies included: Regular, NPH, Lente, Ultralente, & 70/30 (see **APPENDIX**).

The results of these studies, as a whole, strongly indicate that when pramlintide is administered in the same syringe with <u>any</u> insulin product, that both pramlintide and insulin pharmacokinetics can be substantially altered (see **TABLES 12 & 13**). This would then preclude the practice of "mixing" for reasons of compromised pramlintide and insulin efficacy. This conclusion is in line with that of the sponsor. The labeling will clearly indicate that Symlin™ should not be mixed with any insulin product (see **LABELING COMMENTS**).

TABLE 12 – Plasma Pramlintide PK: Symlin™ Mixed with R or NPH Insulin vs. Alone

Parameters	Units	A 30 mcg Symlin + R Insulin	B 30 mcg Symlin + NPH Insulin	C 30 mcg Symlin
C_{max}	pmol/L	37.30 ± 20.38	33.21 ± 20.20	42.43 ± 20.32
AUC_{0-t}	pmol*min/L	1669 ± 1383	1513 ± 1200	1626 ± 1160
T_{max}	min	22.8 ± 9.83	32.3 ± 15.4	18.5 ± 9.07
$t_{1/2}$	min	51.7 ± 14.6	45.4 ± 13.8	47.1 ± 28.6
Mean ± SD				

TABLE 13 – Comparison: Symlin mixed with R or NPH Insulin vs. Alone

Parameters	Units	A vs. C		B vs. C	
		PE	90% CI	PE	90% CI
C_{max}	pmol/L	86.2	73.1 – 99.4	77.2	64.0 – 90.3
AUC_{0-t}	pmol*min/L	98.9	76.7 – 121.1	91.1	68.9 – 113.3
Mean ± SD					

Symlin and Lo/Ovral or Ampicillin

Since pramlintide delays gastric emptying time, what effect does Symlin™ have on orally administered medications?

As previously described, one of pramlintide's primary mechanisms of action is to delay gastric emptying time. Therefore, it is conceivable that if an orally administered drug were administered concomitantly with pramlintide, an interaction may occur such that the object drug's efficacy could be compromised. With this in mind, the sponsor has conducted two drug-drug interaction studies to determine the effect that pramlintide has on an oral contraceptive (OC), Lo/Ovral™ and a relatively acid-stable antibiotic, ampicillin.

Study 137-133 evaluated the effect of pramlintide on the PK of ethinyl estradiol and norgestrel in healthy female subjects receiving Lo/Ovral (30 mcg ethinyl estradiol + 300 mcg norgestrel). In this randomized, two-period crossover design study, 18 females on OC treatment were subcutaneously administered either a placebo injection (lot # 96-0302JE) or a 90 mcg dose of Symlin™ (lot # 96-0503JB – F22 – 0.6 mg/mL) 15 minutes before administration of a single dose of Lo/Ovral™ (Lot # 9978046, expiration: 3/2000).

Results as presented in **TABLE 14** showed no statistically significant differences in the PK profile of ethinyl estradiol on any of the calculated PK parameters. In contrast, the norgestrel component of Lo/Ovral™ did exhibit significant differences when administered with Symlin™ (see **TABLE 15**). The C_{max} for norgestrel was reduced by about 30% and the time to C_{max} was delayed by 45 minutes; AUC was similar between treatments.

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TABLE 14 – Effect of Symlin on Lo/Ovral (ethinyl estradiol) Pharmacokinetics

ETHINYL ESTRADIOL	Lo/Ovral	Lo/Ovral + Symlin	p-value[a]	Ratio or LSMeans Difference	95% CI	
					Low	High
$lnAUC_{0\text{-}24\ hours}$ (pg*min/L)						
LS Means (SE)[a]	10.4 (0.1)	10.4 (0.1)	0.981	100.3%	79.4%	126.6%
GEO LS Means[b]	32990.2	33076.8				
$lnAUC_{0\text{-}inf}$ (pg*min/L)						
LS Means (SE)[a]	10.8 (0.1)	10.9 (0.1)	0.389	109.5%	88.1%	136.0%
GEO LS Means[b]	49544.0	54227.8				
lnC_{max} (pg/L)						
LS Means (SE)[a]	4.6 (0.1)	4.6 (0.1)	0.842	98.5%	84.3%	115.2%
GEO LS Means[b]	101.2	99.7				
T_{max} (min)						
LS Means (SE)[a]	74.0 (14.1)	101.9 (14.1)	0.176	27.9	-13.9	69.7
$t_{½}$ (min)						
LS Means (SE)[a]	413.1 (64.4)	468.8 (64.4)	0.412	55.6	-84.8	196.0

[a] = Based on ANOVA model which includes terms for sequence, subject within sequence, period and treatment
[b] = Geometric means are the antilogs of the means of the natural logarithmic transformed endpoints.

TABLE 15– Effect of Symlin on Lo/Ovral (norgestrel) Pharmacokinetics

NORGESTREL	Lo/Ovral	Lo/Ovral + Symlin	p-value[a]	Ratio or LSMeans Difference	95% CI	
					Low	High
$lnAUC_{0\text{-}24\ hours}$ (ng*min/L)						
LS Means (SE)[a]	7.4 (0.1)	7.4 (0.1)	0.646	98.2	90.4%	106.7%
GEO LS Means[b]	1658.3	1628.6				
$lnAUC_{0\text{-}inf}$ (ng*min/L)						
LS Means (SE)[a]	8.0 (0.1)	8.1 (0.1)	0.188	107.1	96.3%	119.2%
GEO LS Means[b]	3070.2	3288.8				
lnC_{max} (ng/L)						
LS Means (SE)[a]	1.5 (0.1)	1.5 (0.1)	< 0.001	68.9	60.4%	78.5%
GEO LS Means[b]	4.7	3.2				
T_{max} (min)						
LS Means (SE)[a]	68.9 (11.7)	114.2 (11.7)	0.014	45.3	10.9	79.6
$t_{½}$ (min)						
LS Means (SE)[a]	1780 (205)	2054 (205)	0.197	274.9	-162	712

[a] = Based on ANOVA model which includes terms for sequence, subject within sequence, period and treatment
[b] = Geometric means are the antilogs of the means of the natural logarithmic transformed endpoints.

The second drug-interaction study (137-134), which evaluated delayed gastric emptying, was a double-blind, placebo-controlled, single-dose, two-period crossover design study in 12 (11 completed) healthy male and female subjects. Subjects were given either placebo (lot # 96-032JE) plus a single 2 x 250 mg oral dose of ampicillin (batch # 54763 B; expiration 8/2002) or a single 90 mcg subcutaneously administered dose of Symlin™ (lot # 96-0503JB – F22 – 0.6 mg/mL) plus oral ampicillin. Results (**TABLE 16**) show that pramlintide has no significant impact on the AUC or C_{max} of ampicillin, but similar to the Lo/Ovral™ study, T_{max} was increased by approximately one hour.

TABLE 16 – Effect of Symlin on Ampicillin Pharmacokinetics

AMPICILLIN	Ampicillin	Ampicillin + Symlin	p-value[a]	Ratio or LSMeans Difference	95% CI	
					Low	High
$lnAUC_{0\text{-}8\ hours}$ (mcg*min/L)						
LS Means (SE)[a]	6.7 (0.1)	6.6 (0.1)	0.553	95.7	81.5%	112.4%
GEO LS Means[b]	782.3	748.9				
$lnAUC_{0\text{-}Inf}$ (mcg*min/L)						
LS Means (SE)[a]	6.7 (0.1)	6.6 (0.1)	0.559	95.8	81.5%	112.5%
GEO LS Means[b]	792.9	1.6 (0.1)				
lnC_{max} (mcg/L)						
LS Means (SE)[a]	1.5 (0.1)	1.6 (0.1)	0.380	108.3	89.0%	131.9%
GEO LS Means[b]	4.7	5.1				
T_{max} (min)						
LS Means (SE)[a]	110.0 (12.7)	176.2 (12.7)	0.003	66.2	29.1	103.3
$t_{½}$ (min)						
LS Means (SE)[a]	59.1 (3.2)	51.0 (3.2)	0.046	-8.2	-16.2	-0.2

[a] = Based on ANOVA model which includes terms for sequence, subject within sequence, period and treatment
[b] = Geometric means are the antilogs of the means of the natural logarithmic transformed endpoints.

Individually, the results from these studies are relatively unremarkable – especially given that the extent of absorption was not affected by pramlintide in either case. However, both of these agents, ampicillin and Lo/Ovral™, are used chronically and are not dependent upon rapid drug action, as are many oral pain medications, etc. Therefore, as the sponsor has recommended, concomitant medications susceptible to delayed gastric emptying times should be administered at least one-hour before administration of Symlin™.

HUMAN PK – PLASMA/BLOOD

An *in vitro* protein binding study of pramlintide in animal and human blood and plasma. A mean of 37% of pramlintide tracer spiked into whole blood was bound to cells, with the remaining in the unbound in the plasma fraction. Thirty-three percent was bound to soluble plasma components, which leaves approximately 40% total pramlintide available for receptor binding.

HUMAN PK – EX VIVO – Placental Transfer

Placental transfer was evaluated in placentas from normal term vaginal or cesarean section deliveries. Placentas were perfused as an open, non-circulating, system for 90 to 120 minutes and then as a closed, circulating, system for an additional 90 to 120 minutes. Concentrations in the perfusate ranged from 206 to 458 pmol/L.

No detectable pramlintide was detected on the fetal side after 120 minutes of the open-loop perfusion stage. One sample of fetal perfusate, during the closed loop phase, had detectable pramlintide at 60 minutes, but was undetectable at 90 minutes. These *ex vivo* results suggest that fetal exposure to pramlintide *in utero* is low.

PHARMACODYNAMICS

What effect does a morning dose of pramlintide have on the gastric emptying of a lunchtime meal?

Effect of Pramlintide on Gastric Emptying

Delay gastric emptying time is one of the two well described mechanisms of pramlintide action. Gastric emptying, as a pharmacodynamic (PD) endpoint, was formally evaluated in two studies (also see **HUMAN PK – EXTRINSIC – Drug-Drug Interactions**). The first of these studies, 137-118, determined the dose-response relationship of single doses of pramlintide on the rate of gastric emptying of the liquid and solid components of a radio-labeled standardized meal (500 kcal – 55% CHO; 35% fat, 10% protein) and to determine if a dose of pramlintide administered 15 minutes before breakfast has a "carry-over" effect on the emptying of the lunch time meal. This four-way cross-over study in 14 (11 evaluable) type 1 diabetes

patients compared treatments of 30 mcg, 60 mcg, and 90 mcg (lot # 95-0902GB – F21 – 0.3 mg/mL formulation) with placebo (lot # 95-0504GE).

Results of this study showed that when pramlintide is administered 15 minutes before a standardized breakfast consisting of a liquid component, 3-ortho-methyl-glucose (3-OMG) labeled milkshake, and a solid meal, ^{99m}Technetium-amberlite resin labeled pancake, gastric emptying time was significantly delayed compared with placebo. The time to maximum plasma 3-OMG concentration (T_{max}) was increased from about 80 minutes for placebo to 200 minutes for the 90 mcg dose (see **TABLE 17**). The time to achieve half-gastric emptying was similarly increased (see **TABLE 18**). There was no observed "carry-over" effect of the morning pramlintide dose to the lunch time meal.

TABLE 17 – Liquid Meal – Plasma 3-OMG PK Parameters by Treatment (n = 11)

Breakfast (1st meal)	Placebo	30 mcg	60 mcg	90 mcg
$AUC_{0\text{-}240\ min}$ (mmol*min/L)				
Arithmetic mean	41.2	31.0	26.6	29.7
p-value vs placebo	NA	0.0057	0.0005	0.0028
C_{max} (mmol/L)				
Arithmetic mean	0.26	0.20	0.20	0.21
p-value vs placebo	NA	0.0004	0.0008	0.0051
T_{max} (min)				
Arithmetic mean	81.8	170.9	200.0	191.1
p-value vs placebo	NA	0.0006	0.0001	0.0001
Lunch (2nd meal)	**Placebo**	**30 mcg**	**60 mcg**	**90 mcg**
$AUC_{240\text{-}480\ min}$ (mmol*min/L)				
Arithmetic mean	57.9	62.3	60.1	59.9
p-value vs placebo	NA	0.3389	0.6669	0.4977
C_{max} (mmol/L)				
Arithmetic mean	0.33	0.34	0.33	0.33
p-value vs placebo	NA	0.6960	0.7775	0.8379
T_{max} (min)				
Arithmetic mean	318.2	316.4	322.0	308.9
p-value vs placebo	NA	0.8157	0.9782	0.5282
NA = not applicable; p-values from t-tests for multiple comparisons following ANOVA				

TABLE 18 – Solid Meal – Gastric Emptying Parameters by Treatment (n = 11)

Breakfast (1st meal)	Placebo	30 mcg	60 mcg	90 mcg
Half-Emptying Time (min)				
Arithmetic mean	128.6	187.2	200.1	214.5
p-value vs placebo	NA	0.0002	0.0001	0.0001
Lag Time (min)				
Arithmetic mean	32.5	54.4	56.4	70.3
p-value vs placebo	NA	0.0173	0.0130	0.0001
Lunch (2nd meal)	**Placebo**	**30 mcg**	**60 mcg**	**90 mcg**
Half-Emptying Time (min)				
Arithmetic mean	136.9	138.5	140.6	145.4
p-value vs placebo	NA	0.7497	0.7497	0.2590
Lag Time (min)				
Arithmetic mean	34.2	46.5	40.0	48.2)
p-value vs placebo	NA	0.1938	0.5772	0.1353
NA = not applicable; p-values from t-tests for multiple comparisons following ANOVA				

The second gastric emptying study, 137-137, consisted of a two-way crossover design in 10 type 2 diabetes patients that were administered either placebo (lot # 97-0101GE) or a 90 mcg dose of pramlintide (lot # 96-1002JB – F24 – 0.9 mg/mL) 15 minutes prior to a ^{99m}Technetium breakfast (381 kcal – 48% CHO, 26% fat, & 26% protein). The results, as presented in **TABLE 19**, demonstrate that pramlintide significantly increases the half-emptying time and lag time of a solid meal in patients with type 2 diabetes compared with placebo.

TABLE 19 - Solid Meal – Gastric Emptying Parameters

Breakfast	Placebo	90 mcg
Half-Emptying Time (min)		
Arithmetic mean	80.50	127.00
p-value vs placebo	NA	0.0011
Lag Time (min)		
Arithmetic mean	17.00	28.80
p-value vs placebo	NA	0.0232
NA = not applicable; p-values from standard ANOVA for 2x2 crossover		

LABELING

Labeling will be addressed in an amendment to this review that will be submitted after the Advisory Committee Meeting scheduled for July 26, 2001. Attached is the sponsor's proposed labeling.

COMMENTS TO THE SPONSOR

Assay

The immunoenzymetric assay (IEMA) used to detect human plasma pramlintide exhibited reasonable precision and accuracy estimates. However, the samples used in the quality control analysis were sufficiently far enough away from the calibration limits and the lower limit of quantitation as to create some concern about the plasma concentrations that fall between the lower limit of quantitation (LLOQ) and the lowest quality control sample. In addition, the LLOQ appeared to be highly variable between studies. Hence, the reported plasma values that fall on the lower end of the plasma profile curve are not considered reliable.

There is also the issue of using a non-specific assay to characterize the pharmacokinetics of pramlintide. As you have pointed out in your submission, we're actually looking at pramlintide, the pramlintide metabolite(s), and any endogenous material, when describing pramlintide PK. However, the relative contribution of each component is not well described. There is also the potential for additional interference from the human anti-murine antibody (HAMA).

With these issues in mind, the Office of Clinical Pharmacology and Biopharmaceutics strongly suggests that you consider developing a more specific assay for pramlintide that overcomes the described limitations of the IEMA.

Additional Studies

As was made evident during the Advisory Committee meeting held for Symlin™, there are two clinical pharmacology and biopharmaceutics issues that need to be answered through further research:

1. Why is there a difference in the relative bioavailabilities of Symlin™ between patients with type 1 and type 2 diabetes? If this is a body composition issue, then how does one dose Symlin™ in obese patients with type 1 diabetes? Can the differences in bioavailability be overcome by administering Symlin™ at different injection sites e.g., thigh or buttocks as opposed to the abdomen?

2. The timing of dosing for Symlin™ and insulin in relation to the time of food ingestion is an issue that needs to be discussed further. Consider conducting a clinical pharmacology study in patients with type 1 diabetes whereby the timing of the Symlin™, insulin, and food ingestion are optimized for glycemic control and incidence reduction of hypoglycemia. This optimized timing scheme should then be utilized for any further phase 3 studies.

Steven B. Johnson, Pharm.D.
CPB Reviewer

Hae-Young Ahn, Ph.D.
CPB Team Leader

Draft Sign-off: 24-MAY-2001

Final Sign-off: _____

Briefing Date: 01-JUN-2001

Briefing Attendees:
Hae-Young Ahn, Gerald Fetterly, Hank Malinowski, Saul Małozowski, Mei-Ling Chen, Larry Lesko, Sang Chung, Arzu Selen, John Hunt, and Mehul Mehta.

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NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-125

Title of Study: An Open-Label, Randomized, Four-Period Cross-over Study in Normal Volunteers of the Bioavailability of Selected Concentrations of Pramlintide in Two Different Formulations*

Investigators and Study Centers

Publication (Reference): None

Studied Period (Years): September 1996 - April 1997 | **Phase of Development:** 1

Objectives: To determine and compare the bioavailability of similar single subcutaneous (SC) injections of pramlintide (60 µg, 0.1 mL injection volume) formulated at pH 4.7 (formulation F11; treatment A [0.6 mg/mL]) and at pH 4.0 (formulation F22; treatment B [0.6 mg/mL]); to determine the absolute bioavailability of pramlintide formulation F22 (SC = treatment B versus IV = treatment D [0.6 mg/mL]); and to determine the relative contribution of injection volume to the pharmacokinetic behavior of two different concentrations of pramlintide similarly formulated at pH 4.0, formulation F21 (0.3 mg/mL, 0.2 mL SC injection volume): treatment C, and formulation F22 (0.6 mg/mL, 0.1 mL SC injection volume): treatment B.

Methodology: This was a single center, randomized, open-label, single dose, four-way crossover study consisting of four evaluation periods, with a washout period of 1 week between study treatments. Safety data for all 56 subjects are presented in this report, while pharmacokinetic evaluations were done only for subjects at the —— site. Subjects were randomized to one of four possible treatment sequences: ABDC, BCAD, CDBA, or DACB.

Number of Subjects: Fifty-six male subjects between the ages of 18 and 41 years of age, inclusive, were randomized at two study sites. A total of 37 subjects provided sufficient data for all four periods and met all other criteria to be considered evaluable for pharmacokinetic analysis.

Diagnosis and Main Criteria for Inclusion: Healthy, male volunteers who met all inclusion and exclusion criteria.

Test Product, Dose and Mode of Administration, Batch No.: Formulation F11: pramlintide acetate, 0.1 mL (60 µg), pH 4.7, for SC administration, Lot No. 94-0904FB; Formulation F22: pramlintide acetate, 0.1 mL (60 µg), pH 4.0, for SC administration, Lot No. 95-0802GB; Formulation F21: pramlintide acetate, 0.2 mL (60 µg), pH 4.0 for SC administration, Lot No.95-0902GB.

Reference Therapy, Dose and Mode of Administration, Batch No: Formulation F22: pramlintide acetate, (60 µg), pH 4.0, for IV administration, Lot No. 95-0802GB.

Duration of Treatment Four single doses of pramlintide were given with a washout period of 1 week.

** Three formulations of pramlintide were used in this study.*

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Criteria for Evaluation:

Pharmacokinetics: The area under the plasma concentration-time curve from time 0 to infinity ($AUC_{(0-\infty)}$), area under the plasma concentration-time curve from time 0 to 300 minutes ($AUC_{(0-300)}$), concentration maximum (C_{max}), the apparent terminal elimination half-life ($t_{1/2}$), and time of maximum concentration (T_{max}), relative bioavailability ($AUC_{pH4.0}/AUC_{pH4.7}$), and absolute bioavailability (AUC_{SC}/AUC_{IV}) were calculated for each evaluable subject for each treatment.

Safety: Safety was assessed by adverse events, concomitant medications, physical examinations, electrocardiograms, and routine labs including clinical chemistry, hematology, and urinalysis.

Statistical Methods:

Pharmacokinetics: Summary statistics (mean, standard deviation, geometric mean, median, minimum, maximum, and N) for each parameter for each treatment were determined for evaluable subjects. Equivalence of C_{max}, T_{max}, and $AUC_{(0-\infty)}$ values after SC administration of formulations of differing pH were evaluated using 90% (C_{max}, AUC) or 95% (T_{max}) conventional confidence intervals based on an ANOVA model; log$_e$ transformed C_{max}, $AUC_{(0-300)}$, and $AUC_{(0-\infty)}$ values and untransformed T_{max} and $t_{1/2}$ values were evaluated. The ANOVA model included terms for sequence, subjects within sequence, period, treatment, and carry-over. The carry-over effect was tested at the 0.10 significance level and was removed from analyses if not significant.

Safety: Adverse events, vital signs, and clinical laboratory values were summarized by descriptive statistics.

SUMMARY-CONCLUSIONS:

PHARMACOKINETIC RESULTS:

The pharmacokinetic objectives were met. The findings were:

- Mean T_{max} values ranged from 20.3 min to 21.8 min, and $t_{1/2}$ ranged from 43.6 min to 49.3 min for the three SC formulations. Pramlintide was eliminated following IV administration, with a $t_{1/2}$ of 34.1 min.
- Doubling the SC injection volume from 0.1 mL to 0.2 mL produced negligible changes in C_{max}, $AUC_{(0-\infty)}$, and T_{max} values for the F21 (0.2 mL) formulation relative to the F22 (0.1 mL) formulation. Mean C_{max} values were 80.3 pmol/L and 90.0 pmol/L, $AUC_{(0-\infty)}$ values were 6334 pmol•min/L and 6639 pmol•min/L, and T_{max} values were 21.8 min and 20.3 min for the F21 and F22 formulations, respectively.
- A higher formulation pH (4.7 vs 4.0) substantially decreased both C_{max} and $AUC_{(0-\infty)}$ parameters following SC administration.
- Formulation pH apparently had no effect on the mean T_{max} parameter following SC administration. The elimination half-life ($t_{1/2}$) was consistent among SC treatments but was somewhat shorter following IV administration (34.1 min).
- Absolute bioavailability of SC-administered pramlintide (pH 4.0 formulation) was between 38% and 40% compared to IV administration.

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events:

Adverse events were reported separately for each treatment period (up to 300 minutes postdose) and the subsequent post-treatment period (300 minutes postdose up until the next dose period). The incidence of adverse events occurring on the day of treatment was 37.5%, overall. Incidences were similar following either of the 0.1 mL SC injection volume treatments (treatment A, 3.7%; treatment B, 3.8%), slightly higher following the 0.2 mL SC injection volume treatment (treatment C, 7.5%), and considerably higher following the IV administration of pramlintide (treatment D, 36.0%).

The most common adverse event for all treatment periods was nausea. There was no nausea reported between treatments. The incidence of nausea was 3.7%, 1.9%, 1.9%, and 28.0% for treatments A, B, C, and D, respectively. Other events reported on the day of treatment with SC pramlintide included headache, diarrhea, and abdominal pain. Events other than nausea reported on the day of treatment with IV pramlintide included enlarged abdomen, hot flushes, dizziness, dysesthesia, headache, paresthesia, flatulence, dry mouth, vomiting, and taste perversion. Taste perversion had the second highest incidence among adverse events occurring following IV administration of pramlintide.

There were no withdrawals from the study because of adverse events, no serious adverse events, and no deaths.

Clinical Laboratory Values:

There were no clinical laboratory results that were of clinical concern or suggested a relationship to treatment with pramlintide.

CONCLUSION:

- The administration of single 60-µg SC and IV doses of pramlintide to healthy male subjects was well tolerated in this study.
- C_{max} and $AUC_{(0-\infty)}$ were substantially decreased with a higher formulation pH (4.7).
- The absolute bioavailability of the SC-administered, pH 4.0, formulation was between 38% and 40% compared to IV administration.
- The relative contribution of injection volume to the pharmacokinetic behavior of two different concentrations of pramlintide, both similarly formulated at pH 4.0, was negligible.

Date of the report: 17 Mar 00

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection	Volume:	
NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	Page:	

Protocol No.: 137-142

Title of Study: An Open-Label, Randomized, Two-Period Cross-Over Study in Healthy Volunteers of the Bioequivalence of Two Different Formulations and Dosage Forms of Pramlintide (AC137)

Investigators and Study Centers: [redacted]

Publication (Reference): None

Studied Period (Years): November 1999 - December 1999

Phase of Development: 1

Objectives: To determine the bioequivalence of pramlintide (AC137) administered by two different delivery systems (a pen-cartridge system using a 1.0 mg/mL formulation and a vial-syringe system using a 0.6 mg/mL formulation).

Methodology: This was a single-center, open-label, randomized, two-period crossover study consisting of two evaluation periods with a period of 24 hours between each dosing event, designed to evaluate the bioequivalence of vial and cartridge dosage forms of pramlintide. Subjects received the following single dose treatments (A and B) of pramlintide on alternating days as scheduled according to a balanced randomization scheme.

A. 60 µg in a 1.0 mg/mL formulation in cartridge dosage form for pen administration.
B. 60 µg in a 0.6 mg/mL formulation in vial dosage form for syringe administration.

Number of Subjects: Thirty subjects (20 female, 10 male; mean age 42.4 years) were enrolled in this study. All 30 subjects completed all study procedures and study visits (screening, Day 0, Period 1 [Day 1], Period 2 [Day 2], and study exit on Day 2). Evaluable subjects were defined as subjects who satisfied all criteria for entry into the study, completed each of the two treatment periods, and had sufficient concentration data from both dosing periods to allow pharmacokinetic analysis.

Diagnosis and Main Criteria for Inclusion: Normal, healthy males or females, aged between 18 and 65 years.

Test Form, Dose and Mode of Administration, Batch No.: Cartridge, pramlintide 1.0 mg/mL, 0.06 mL per subject, pen administration of subcutaneous injection, Lot number: (AC137-F26) 97-0402KB.

Duration of Treatment: Two single-dose periods with at least a 24-hour washout period between dosing.

Reference Form, Dose and Mode of Administration, Batch No: Vial, pramlintide 0.6 mg/mL, 0.10 mL per subject, syringe administration of subcutaneous injection, Lot number: (AC137-F22) 97-1204JB.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Criteria for Evaluation:

Phamacokinetics: The primary pharmacokinetic measures for demonstrating bioequivalence were C_{max} and $AUC_{(0\text{-}300\ min)}$. Since minimum detectable concentrations at the end of the concentration-time profile, and concentrations at –15, -5, and 0 minutes were set to zero, $AUC_{(0\text{-}300\ min)}$ is equivalent to $AUC_{(0\text{-}t)}$.

Safety: Safety and tolerability were assessed throughout the study period by monitoring adverse events, vital signs, and clinical laboratory tests.

Statistical Methods:

Pharmacokinetics: Bioequivalence between the pen-cartridge and syringe-vial delivery systems was based on an ANOVA of natural log transformed C_{max} and $AUC_{(0\text{-}300\ min)}$ parameters. Ninety percent confidence intervals were calculated for the ratios (pen-cartridge/vial-syringe) of the geometric means. Bioequivalence was concluded if the 90% confidence intervals fell within (80%, 125%).

Safety: Adverse events, clinical laboratory values, and vital signs were presented using descriptive statistics.

SUMMARY - CONCLUSIONS:

PHARMACOKINETIC RESULTS: The pen-cartridge system using a 1.0 mg/mL pramlintide formulation and the vial-syringe system using a 0.6 mg/mL pramlintide formulation were bioequivalent: the 90% confidence intervals for the ratios between geometric means for C_{max}, $AUC_{(0\text{-}300\ min)}$, and $AUC_{(0\text{-}\infty)}$ all fell within 80%-125%. In addition, the median T_{max} and mean $t_{1/2}$ remained relatively unchanged.

Parameter	Treatment A Mean[b]	Treatment B Mean[b]	Treatment A/Treatment B[c] Ratio (%)	90% C.I.[d] (%)	P-value[e] Treatment
C_{max}[a] (pmol/L)	67.26	62.07	108.37	(100.0, 117.4)	0.0986
$AUC_{(0\text{-}300\ min)}$[a] (pmol*min/L)	4845.30	4678.83	103.56	(93.3, 115.0)	0.5742
$AUC_{(0\text{-}\infty)}$[a] (pmol*min/L)	5673.14	5672.87	100.00	(90.5, 110.5)	0.9993
$t_{1/2}$ (min)	52.79	59.40	88.89	(75.9, 101.8)	0.1554
T_{max} (min)	20 00	20.00			0.1226

Note: Treatment A: 60 µg pen-cartridge system using a 1.0 mg/mL formulation. Treatment B: 60 µg vial-syringe system using a 0.6 mg/mL formulation.

[a] Parameters were natural log-transformed before analysis.

[b] Geometric means (anti-log of the means of the logs) were analyzed for the natural log-transformed parameters. Arithmetic means are presented for $t_{1/2}$ and median values presented for T_{max}.

[c] Ratio of geometric means calculated as Treatment A/ Treatment B (test/reference).

[d] 90% confidence interval of the geometric means ratio Treatment A/Treatment B.

[e] P-value from ANOVA (including terms for sequence, subject-within-sequence, period and treatment) for testing treatment differences. For T_{max}, the p-value from the Wilcoxon signed-rank test is presented.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events: Of the 30 randomized subjects, seven (23.3%) experienced seven adverse events during the study. The overall incidence of adverse events was similar between treatments (10.0%-Treatment A; 13.3%-Treatment B). Headache (6.7%) and nausea (6.7%) were the most commonly occurring adverse events. All adverse events were assessed as mild in intensity by the Investigator.

Dyspesia (3.3%) and nausea (6.7%) occurred only during Treatment A, while dizziness (3.3%), dreaming abnormal (3.3%), and headache (6.7%) occurred only during Treatment B.

Six (20.0%) subjects experienced six adverse events that were possibly or probably related to study drug according to the Investigator. The overall incidence of possibly or probably related adverse events was the same between treatments (10.0%-Treatment A; 10.0%-Treatment B). Possibly or probably related adverse events were nausea (6.7%, Treatment A), headache (6.7%, Treatment B), dizziness (3.3%, Treatment B), and dyspepsia (3.3%, Treatment A).

Deaths: None

Serious Adverse Events: None

Clinical Laboratory Values: No clinically meaningful or unexpected changes were reported in clinical laboratory variables.

Vital Signs and Physical Examinations: No clinically meaningful or unexpected changes were reported in vital signs or physical examinations.

CONCLUSIONS:

Pramlintide administered subcutaneously by two different delivery systems (a pen-cartridge system using a 1.0 mg/mL formulation and a vial-syringe system using a 0.6 mg/mL formulation) was shown to be bioequivalent.

Pramlintide administered subcutaneously by two different delivery systems (a pen-cartridge system using a 1.0 mg/mL formulation and a vial-syringe system using a 0.6 mg/mL formulation) appeared to be safe and well-tolerated.

Date of the report: 02 June 2000

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-126

Title of Study: An Open-Label, Randomized, Four-Period Cross-Over Study of the Proportionality of Four Subcutaneous Doses of Pramlintide (AC137) Administered at Constant Volume in Normal Volunteers

Investigators and Study Centers: [redacted]

Publication (Reference): None

Studied Period (Years): October 1996 to March 1997

Phase of Development: I

Objective: Determine the profiles and comparative relationship of plasma pramlintide concentrations after subcutaneous administration of single, consecutive randomized 0.2 mL doses of each of three different strengths (0.3, 0.6, and 9.0 mg/mL) of the Phase 3 clinical trial formulation. Doses to be assessed were 30, 60, 90, and 120 µg. The 120 µg was attained by administering 0.2 mL of the 0.6 mg/mL presentation. Thirty, 60, and 90 µg doses were achieved by administering 0.1 mL of 0.3, 0.6, and 9.0 mg/mL presentations respectively, and 0.1 mL of placebo.

Methodology: This was a randomized, single center, open-label, four-way cross-over study consisting of four evaluation periods with a washout period of 1 week between each dosing event. Each subject received four different doses (30, 60, 90, and 120 µg) of pramlintide administered as subcutaneous injections. Thirty, 60, and 90 µg doses were achieved by administering 0.1 mL of 0.3, 0.6, or 0.9 mg/mL concentrations respectively, combined in the same syringe with 0.1 mL of placebo. The 120 µg dose was attained by administering 0.2 mL of the 0.6 mg/mL concentration.

Number of Subjects: Forty healthy male subjects between the ages of 18 and 40 years, inclusive, were randomized to study medication. A total of 38 subjects completed all four evaluation periods of the study. Thirty-six subjects were evaluable for pharmacokinetic analysis. All 40 randomized subjects were included in the safety analyses.

Diagnosis and Main Criteria for Inclusion: Normal, healthy male subjects who met all inclusion and exclusion criteria were eligible for study inclusion.

Test Product, Dose, and Mode of Administration, Batch No.: Pramlintide acetate 30 µg: 0.1 mL of AC137 Formulation code F21 (Lot No. 96-0201GB) and 0.1 mL of placebo (Lot No. 95-0504GE); Pramlintide acetate 60 µg: 0.1 mL of AC137 Formulation code F22 (Lot No. 96-0503JB) and 0.1 mL of placebo (Lot No. 95-0504GE); Pramlintide acetate 90 µg: 0.1 mL of AC137 Formulation code F24 (Lot No. 96-0506JB) and 0.1 mL of placebo (Lot No. 95-0504GE); Pramlintide acetate 120 µg: (0.2 mL of AC137 Formulation code F22 (Lot No. 96-0503JB). All administered by subcutaneous injection.

Duration of Treatment: Four single, subcutaneous doses were scheduled for each subject with a washout period of 1 week between doses.

Reference Therapy, Dose and Mode of Administration, Batch No: None

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	Volume: Page:	

Criteria for Evaluation:

Pharmacokinetic: The pharmacokinetic parameters used to summarize each subject's plasma pramlintide concentration profiles were as follows: concentration maximum (C_{max}), time of maximum concentration (T_{max}), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$), and half-life ($t_{1/2}$).
Safety: The following parameters were assessed throughout the study period to assess safety: adverse events; concomitant medications; routine labs including clinical chemistry, hematology and urinalysis; vital signs; physical examinations and electrocardiograms.

Statistical Methods:

Pharmacokinetic (Pramlintide): $AUC_{(0\text{-}300)}$, $AUC_{(0-\infty)}$, and C_{max} were dose-normalized and then were log-transformed. The log-transformed $AUC_{(0\text{-}300)}$, $AUC_{(0-\infty)}$, and C_{max} were analyzed by the ANOVA model, which included terms for treatment, period, sequence, carryover, and subject within sequence. Sequence was tested by the subject-within-sequence error term, while period, carryover, and treatment were tested by the intra-subject error term. The carryover effect was tested at the 0.05 significance level. Ninety percent confidence intervals were constructed for the differences in the least squares means of the log-transformed dose-normalized AUC and C_{max} data between each pair of the two doses. Dose-independent pharmacokinetic parameters (T_{max} and $t_{1/2}$) were analyzed similarly without any transformation, and the 95% confidence intervals were calculated for the pairwise differences between the doses.
Summary statistics, including mean, geometric mean, standard error of the mean (SEM), median, minimum, maximum, and N were calculated for $AUC_{(0\text{-}300)}$, $AUC_{(0-\infty)}$ and C_{max}, T_{max}, and $t_{½}$ for all evaluable subjects within each treatment.

Safety: Adverse events, vital signs, and clinical laboratory values are presented using descriptive statistics.

SUMMARY - CONCLUSIONS:

PHARMACOKINETIC RESULTS:

- Pramlintide concentrations increased with increasing subcutaneous dose over the dose range of 30 to 120 µg.
- $AUC_{(0-\infty)}$ for baseline-adjusted pramlintide increased with increasing dose in a nearly dose-proportional manner.
- C_{max} for baseline-adjusted pramlintide increased with increasing dose in a dose-proportional manner.
- The pharmacokinetic parameters T_{max} and elimination half-life ($t_{1/2}$) appeared to be independent of the pramlintide doses evaluated in the present study.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events: Of the 40 randomized subjects, 17 (42.5%) subjects experienced a total of 35 adverse events (treatment periods + post-treatment periods). The most commonly occurring adverse events were headache (27.5%), nausea (10.0%) and pharyngitis (10.0%). The majority of headaches were reported during the post-treatment periods and were not treatment-related.

No relationship was observed between the dose of pramlintide and incidence of adverse events during the 300-minute treatment period. The highest incidence of adverse events was reported by Treatment B (60 µg; 10.3%), followed by Treatment C (90 µg; 7.7%) and Treatment D (120 µg; 5.1%). No incidence of adverse events was reported for Treatment A (30 µg; 0.0%). Headaches occurred in 5.1% of subjects receiving Treatment B (60 µg), and 2.6% of subjects receiving Treatments C (90 µg) and D (120 µg). The incidence of nausea ranged from 2.6% for subjects receiving Treatment D (120 µg) to 5.1% for subjects receiving Treatment B (60 µg) and Treatment C (90 µg).

Eleven (27.5%) subjects reported 24 events that were considered possibly or probably treatment-related by the investigator. The most commonly occurring of these were headache (17.5%) and nausea (10.0%). During the pramlintide treatment periods, only two headaches and five nausea events were considered treatment-related. The same subject (5026) experienced three of the five treatment-related nausea events during different treatment periods (B, C, and D). Other treatment-related adverse events included dizziness, asthenia, hot flush, rash and flushing.

Deaths: No deaths were reported in this study.

Serious Adverse Events: No serious adverse events occurred during this study.

Clinical Laboratory Values: No clinically meaningful or unexpected changes were reported in clinical laboratory variables.

Vital Signs, Physical Examinations and ECGs: Pramlintide treatment appeared to have no effect on vital signs, physical examinations, and electrocardiogram measurements.

CONCLUSION:

Plasma pramlintide AUC and C_{max} increased with single subcutaneous pramlintide doses (30, 60, 90, and 120 µg) in a dose-proportional manner, while T_{max} and $t_{1/2}$ were generally independent of dose.

Subcutaneous administration of single, consecutive, randomized 0.2 mL doses (30, 60, 90, and 120 µg) of pramlintide appeared to be well tolerated. No unexpected findings or dose trends were observed for subjects receiving pramlintide.

Date of the report: 14 Apr 2000

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)
Protocol No.: 137-127		
Title of Study: An Open-Label, Single-Dose, Pharmacokinetic Study of Pramlintide in Type I Diabetics With Renal Impairment		
Investigators and Study Centers: [redacted]		
Publication (Reference): None		
Studied Period (Years): August 1997 - May 1998		**Phase of Development:** 1
Objectives: 1) To determine the effect of different degrees of renal impairment on the plasma concentration profiles and pharmacokinetics of pramlintide. 2) To determine the safety of administering pramlintide to subjects with different degrees of renal impairment.		
Methodology: This was an open-label, single dose, parallel-group study of the effects of renal impairment on the pharmacokinetics of pramlintide in subjects with type 1 diabetes mellitus. Following completion of study procedures and a baseline iothalamate and PAH evaluation, subjects received a single subcutaneous dose of pramlintide (60 µg) and blood samples for pharmacokinetic evaluation were collected during a 10-hour period.		
Number of Subjects: Twenty-one subjects (5 female, 16 male; mean age 41.0 years) were enrolled in this study. All 21 subjects completed all study procedures and study visits (screening, baseline iothalamate and PAH evaluations, pharmacokinetic evaluations, and study termination). Evaluable subjects were defined as those who satisfied all criteria for entry into the study, finished the baseline iothalamate and PAH evaluation, and had sufficient blood samples collected during the pharmacokinetic evaluation period to allow pharmacokinetic analysis. All subjects that received study medication were evaluable and were also included in the safety analyses.		
Diagnosis and Main Criteria for Inclusion: Healthy and ambulatory, males or females, aged 18 years or older, with type 1 diabetes mellitus requiring treatment with insulin for at least 12 months prior to the screening visit. Renal function fell into one of four categories based on creatinine clearance: Group I: creatinine clearance ≥80 mL/min; Group II: creatinine clearance 50-80 mL/min; Group III: creatinine clearance 30-50 mL/min; and Group IV: creatinine clearance <30 mL/min.		
Test Form, Dose and Mode of Administration, Batch No.: Pramlintide 0.6 mg/mL, 0.10 mL per subject, administered subcutaneously, Lot number: 95-0802GB (AC137-F22).		
Duration of Treatment: One single-dose period followed by a ten hour pharmacokinetic evaluation.		
Reference Form, Dose and Mode of Administration, Batch No: Not applicable.		

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Criteria for Evaluation:

Pharmacokinetics: The pharmacokinetic measures for determining the effect of different degrees of renal impairment on the plasma concentration profiles and pharmacokinetics of pramlintide were C_{max}, T_{max}, $AUC_{(0-\infty)}$, $t_{1/2}$, apparent clearance (CL/F), and cumulative urinary excretion (CUE).

Safety: Safety was assessed throughout the study by monitoring adverse events, clinical laboratory tests, vital signs, physical examinations, and electrocardiograms.

Statistical Methods:

Pharmacokinetics: Descriptive statistics were presented for all the pharmacokinetic parameters. Individual concentration profiles and mean concentration profiles were plotted within each creatinine clearance category. Individual concentration profiles were also plotted in semi-log scale. The linear relationship between the pharmacokinetic parameters and renal function was examined to determine whether dose adjustment was necessary for subjects with different degrees of renal impairment. Regression models were run to predict each of the above pharmacokinetic parameters based on the measures of renal function.

Safety: Adverse events, clinical laboratory values, and vital signs were presented using descriptive statistics.

SUMMARY - CONCLUSIONS:

PHARMACOKINETIC RESULTS: Similar mean plasma pramlintide concentration profiles were observed among the four creatinine clearance groups following the subcutaneous administration of a single 60 µg dose of pramlintide. As shown below, there was no significant correlation between renal function (glomerular filtration rate [creatinine and iothalamate clearances] and renal plasma flow [PAH clearance]) and the pharmacokinetics of pramlintide.

	24-Hour Creatinine Clearance		Iothalamate Clearance		PAH Clearance	
PK Parameter	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
C_{max} (pmol/L)	0.11	0.641	-0.11	0.627	-0.07	0.779
T_{max} (min)	0.22	0.343	0.13	0.573	0.11	0.622
$AUC_{(0-\infty)}$ (pmol•min/L)	0.07	0.768	-0.29	0.198	-0.23	0.321
$t_{1/2}$ (min)	-0.15	0.523	-0.17	0.468	-0.00	0.990
CL/F (L/hr)	-0.23	0.324	0.16	0.487	-0.09	0.695
CUE (mL)	0.10	0.651	0.05	0.815	0.25	0.269

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection	Volume: Page:	
NAME OF ACTIVE INGREDIENT(S): pramlintide acetate		

SAFETY RESULTS:

Adverse Events: Of the 21 randomized subjects, nine (42.9%) experienced a total of 15 adverse events during the study. The most commonly occurring adverse events were headache (23.8%), nausea (9.5%), and dizziness (9.5%). The majority of adverse events were assessed as mild in intensity. Six (28.6%) subjects experienced a total of 11 adverse events that were considered possibly or probably related to study drug administration (4-headaches, 2-nausea, 2-dizziness, 1-asthenia, 1-diarrhea, and 1-sweating increased).

There did not appear to be any correlation between degree of renal impairment and incidence of adverse events. The overall incidence of adverse events was highest for Group IV (66.7%) and Group II (62.5%), followed by Group I (33.3%). No adverse events were reported for Group III.

Deaths: None

Serious Adverse Events: None

Hypoglycemic Episodes: Four (19.0%) subjects recorded a hypoglycemic episode during the study; three (37.5%) subjects from Group II and one (25.0%) subject from Group III. Glucose concentrations were as follows: Group II-subject 0001 (88 mg/dL); subject 0009 (165 mg/dL); and subject 0029 (68 mg/dL); Group III-subject 0004 (57 mg/dL). All four episodes were resolved by administering a snack, juice, or oral glucose.

Clinical Laboratory Values: There were no unexpected changes reported in clinical laboratory values. The most frequent potentially clinically important laboratory values were: elevated urine glucose, decreased hematocrit, decreased hemoglobin, elevated creatinine, elevated blood glucose, and elevated urea. All of these were probably not attributed to pramlintide, but rather to the underlying disease (renal impairment and diabetes mellitus type 1) of the study population. Additionally, there was no correlation between the degree of renal impairment and incidence of potentially clinically important laboratory values.

Vital Signs, Physical Examinations, and Electrocardiograms: There were no clinically meaningful or unexpected changes reported in vital signs, physical examinations, or electrocardiograms.

CONCLUSIONS:

- Impaired renal function had no significant influence on pramlintide pharmacokinetics; therefore, no dosing adjustment is required for pramlintide when administered to subjects with impaired renal function.
- The subcutaneous administration of a 60 µg dose of pramlintide in subjects with different degrees of renal impairment appeared to be consistently safe and well-tolerated.

Date of the report: 11 July 2000

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-133

Title of Study: A Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Two-Period Crossover Study to Evaluate the Effect of Pramlintide on the Pharmacokinetics of Ethinyl Estradiol and Norgestrel in Healthy Female Subjects Receiving the Oral Contraceptive Agent Lo/Ovral®

Investigator and Study Center: [redacted]

Publication (Reference): None

Studied Period (Years): May 1998 – June 1998 | **Phase of Development:** I

Objectives: To evaluate the effect of pramlintide on the pharmacokinetics of ethinyl estradiol and norgestrel in healthy female subjects receiving the oral contraceptive agent Lo/Ovral (30 µg of ethinyl estradiol and 300 µg of norgestrel). To evaluate the safety and tolerability of concomitant administration of pramlintide and the oral contraceptive agent Lo/Ovral.

Methodology: This was a single-center, randomized, double-blind, placebo-controlled, single-dose, 2-period crossover study with at least a 2-week washout between doses in Period 1 and Period 2. Subjects were randomized to one of two treatment sequences, A:B or B:A, where A=placebo and B=pramlintide.

Number of Subjects: 18

Diagnosis and Main Criteria for Inclusion: Subjects were healthy, non-smoking adult females (18-38 years inclusive) with a history of regular menstrual cycles, who were not currently using oral or injectable contraceptives.

Test Product, Dose and Mode of Administration, Batch No.: 90 µg (0.15 mL of a 0.6 mg/mL solution) pramlintide (AC0137-F22; Lot no. 96-0503JB) administered by subcutaneous injection, followed by a single tablet of Lo/Ovral (30 µg ethinyl estradiol and 300 µg of norgestrel, Wyeth Ayerst, Lot #9978046, expiration 3/00) administered orally 15 minutes later.

Duration of Treatment: Two single doses of study medication (active or placebo) were given with a 2-week washout period between doses. Subjects remained in-house on Days 1-3 of each dosing period and returned on Days 4-9 for blood sampling. Study exit procedures were done on Day 9 of Period 2.

Reference Therapy, Dose and Mode of Administration, Batch No: 0.15 mL placebo (Lot no. 96-0302JE) administered by subcutaneous injection, followed by a single tablet of Lo/Ovral (30 µg ethinyl estradiol and 300 µg of norgestrel, Wyeth Ayerst, Lot #9978046, expiration 3/00) administered orally 15 minutes later.

Criteria for Evaluation:

Pharmacokinetics: Blood samples were collected up to 168 hours after Lo/Ovral dosing during each study period for the determination of ethinyl estradiol and norgestrel plasma concentrations. The C_{max}, $t_{1/2}$ T_{max}, $AUC_{0\text{-}24h}$, and $AUC_{0\text{-}inf}$ of each component for the 2 treatments, i.e., with and without concomitant pramlintide administration, were compared.

Safety: Safety and tolerability were assessed throughout the study by monitoring adverse events and by clinical laboratory evaluations, vital signs, physical examinations, and ECGs.

Statistical Methods:

Pharmacokinetics: Pharmacokinetic parameters were analyzed for evaluable subjects by ANOVA, and the 95% confidence intervals were calculated for the ratios or differences between the least squares means or geometric means for the two treatments.

Safety: Selected safety data were summarized for all randomized subjects (intent-to-treat).

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection	Volume:	
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NAME OF ACTIVE INGREDIENT(S): pramlintide acetate		

SUMMARY – CONCLUSIONS:

PHARMACOKINETIC RESULTS:

There were no statistically significant differences in the pharmacokinetic parameters of ethinyl estradiol after pramlintide, compared with placebo, as seen below, based on an analysis excluding potential outlier concentrations.

Parameter (units)	Treatment	LSMean[1] (SE) {Geometric LSMean}[2]	p-value[1]	Ratio or difference of the LSMeans	95% CI of the ratio or difference
Ethinyl estradiol					
ln AUC(0-24h) (pg*min/mL)	Placebo	10.4 (0.1) {32990.2}	0.981	100.3%	(79.4%, 126.6%)
	Pramlintide	10.4 (0.1) {33076.8}			
ln AUC(0-inf) (pg*min/mL)	Placebo	10.8 (0.1) {49544.0}	0.389	109.5%	(88.1%, 136.0%)
	Pramlintide	10.9 (0.1) {54227.8}			
ln C(max) (pg/mL)	Placebo	4.6 (0.1) {101.2}	0.842	98.5%	(84.3%, 115.2%)
	Pramlintide	4.6 (0.1) {99.7}			
t(1/2) (min)	Placebo	413.1 (64.4)	0.412	55.6	(-84.8, 196.0)
	Pramlintide	468.8 (64.4)			
T(max) (min)	Placebo	74.0 (14.1)	0.176	27.9	(-13.9, 69.7)
	Pramlintide	101.9 (14.1)			

ln=natural logarithm

1 Based on an ANOVA model which included terms for sequence, subject-within-sequence, period, and treatment

2 Geometric means are the antilogs of the means of the natural logarithmic transformed endpoints.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	Volume: Page:	

C_{max} was decreased and T_{max} and $t_{½}$ were delayed after pramlintide treatment, as seen below. The overall exposure to norgestrel, as reflected in AUC_{0-inf}, was not different after co-administration of Lo/Ovral with pramlintide.

Parameter (units)	Treatment	*LSMean[1] (SE) {Geometric LSMean}[2]*	p-value[1]	*Ratio or difference of the LSMeans*	*95% CI of the ratio or difference*
Norgestrel					
ln AUC(0-24h) (ng*min/mL)	Placebo	7.4 (0.1) {1658.3}	0.646	98.2%	(90.4%, 106.7%)
	Pramlintide	7.4 (0.1) {1628.6}			
ln AUC(0-inf) (ng*min/mL)	Placebo	8.0 (0.1) {3070.2}	0.188	107.1%	(96.3%, 119.2%)
	Pramlintide	8.1 (0.1) {3288.8}			
ln C(max) (ng/mL)	Placebo	1.5 (0.1) {4.7}	<0.001	68.9%	(60.4%, 78.5%)
	Pramlintide	1.2 (0.1) {3.2}			
t(1/2) (min)	Placebo Pramlintide	1779.5 (204.8) 2054.3 (204.8)	0.197	274.9	(-162, 712.0)
T(max) (min)	Placebo Pramlintide	68.9 (11.7) 114.2 (11.7)	0.014	45.3	(10.9, 79.6)

ln=natural logarithm
1 Based on an ANOVA model which included terms for sequence, subject-within-sequence, period, and treatment
2 Geometric means are the antilogs of the means of the natural logarithmic transformed endpoints.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): *pramlintide acetate*	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events: In this cross-over study, similar safety profiles were observed when pramlintide or placebo was co-administered with Lo/Ovral. The number of subjects reporting adverse events was similar for both treatments, with 8 (44.4%) subjects reporting adverse events during treatment with pramlintide and 9 (52.9%) during treatment with placebo. Headache was the most commonly reported adverse event, reported by 3 (16.7%) and 5 (29.4%) subjects in the pramlintide and placebo treatment periods, respectively. The second most commonly reported adverse event was nausea, reported by 4 subjects (22.2%) receiving pramlintide and zero subjects receiving placebo. However, 1 subject in each treatment group (pramlintide and placebo) experienced vomiting. There were no hypoglycemic events.

Deaths: There were no deaths.

Serious Adverse Events: There were no serious adverse events.

Clinical Laboratory Values: There were no laboratory parameters that appeared to be adversely affected by pramlintide when co-administered with Lo/Ovral.

Vital Signs and Physical Examination: There were no vital signs results that would suggest an adverse effect of pramlintide co-administered with Lo/Ovral.

CONCLUSIONS:

- Pramlintide administered as a single 90-µg dose concomitantly with the oral contraceptive agent Lo/Ovral (30 µg ethinyl estradiol and 300 µg of norgestrel) was well-tolerated.
- There were no statistically significant differences in the pharmacokinetic profile of ethinyl estradiol for $AUC_{0\text{-}24h}$, $AUC_{0\text{-}inf}$, C_{max}, $t_{1/2}$, and T_{max} after treatment with pramlintide compared with placebo.
- The C_{max} for norgestrel was reduced by approximately 30% and the T_{max} was delayed by 45 minutes after pramlintide treatment. These differences were statistically significant. There were no statistically significant differences in $AUC_{0\text{-}24h}$, $AUC_{0\text{-}inf}$, or $t_{1/2}$ after treatment with pramlintide or placebo, suggesting that total exposure to norgestrel is not affected by pramlintide. The C_{max} for norgestrel observed after treatment with pramlintide is consistent with therapeutic concentrations observed after norgestrel treatment as described in the literature.

Date of the report: 26 July 00

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-134

Title of Study: A Randomised, Double-Blind, Placebo-Controlled, Single-Dose, Two-Period Crossover Study to Determine the Effect of Pramlintide on the Pharmacokinetics of Ampicillin in Healthy Subjects

Investigator and Study Center: [redacted]

Publication (Reference): None

Studied Period (Years): 1998 | **Phase of Development:** I

Objectives: The primary objective of this study was to evaluate the effect of pramlintide on the pharmacokinetics of ampicillin in healthy subjects. The C_{max}, T_{max}, $t_{½}$ and $AUC_{(0\text{-}8h)}$ of ampicillin from the two treatments, i.e., with and without concomitant pramlintide administration, were to be compared. The secondary objective was to evaluate the safety and tolerability of single-dose concomitant administration of pramlintide and ampicillin. During the course of the statistical analysis, a decision was made to calculate $AUC_{(0\text{-}inf)}$ as well as $AUC_{(0\text{-}8h)}$.

Methodology: This was a single-center, randomized, double-blind, placebo-controlled, single-dose, two-period crossover study with at least a seven day washout period between doses in Period 1 and Period 2. Subjects were to be randomized to one of two treatment sequences (A:B or B:A), where A was placebo and B was pramlintide (90 µg, 0.6 mg/mL).

Number of Subjects: 12 (5 males and 7 females)

Diagnosis and Main Criteria for Inclusion: Subjects were to be fasted, healthy male (at least four) and female (at least four) adults (18 to 55 years of age, inclusive), with no history of cardiovascular, renal, pulmonary, hematological, CNS or GI disease. Females were to be surgically sterile, post-menopausal or practicing appropriate contraception to ensure that pregnancy would not occur during the study. All subjects were to be drug and alcohol free, have no history of penicillin allergy and were not to be taking any other concomitant medication.

Test Product, Dose and Mode of Administration, Batch No.: Pramlintide (90 µg, 0.15 mL of a 0.6 mg/mL solution) injected subcutaneously (Lot no. 96-0503JB).

Duration of Treatment: Two single doses of the study medication (pramlintide or placebo) were given with at least a 7-day washout period between doses.

Reference Therapy, Dose and Mode of Administration, Batch No: Placebo (0.15 mL) injected subcutaneously (Lot no. 96-0302JE). Ampicillin (as the trihydrate, [redacted] 2x250-mg capsules, [redacted], Batch No.: 54763 B. Expiry date: August 2002). Two capsules/dose were administered orally 15 minutes after subcutaneous pramlintide or placebo injection.

Criteria for Evaluation:

Pharmacokinetics: Blood samples were collected from each subject at 0 min (pre-dose), 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 5 h, 6 h, and 8 h after the ampicillin dose in each period to determine the concentration of the antibiotic in the isolated plasma. Initially, the variables to be analyzed were $AUC_{(0\text{-}8h)}$, C_{max}, $t_{1/2}$ and T_{max} for ampicillin. The highest plasma concentration (C_{max}) and the time to C_{max} (T_{max}) for each subject were determined from the observed data. The area under the plasma concentration-time curve for ampicillin for the time interval 0 min (pre-dose) to 8h post dose ($AUC_{(0\text{-}8h)}$) was estimated from the observed data, by applying the linear trapezoidal rule and subsequently used to calculate the $AUC_{(0\text{-}inf)}$.

Safety: Safety and tolerability were assessed throughout the course of the study by monitoring adverse events, clinical laboratory evaluations, vital signs, and physical examinations.

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume:	(FOR NATIONAL AUTHORITY USE ONLY)
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Statistical Methods:

Pharmacokinetics: Ampicillin pharmacokinetic parameters were to be analyzed for all evaluable subjects by ANOVA and the 95% confidence intervals were to be calculated for the ratios or differences of the adjusted means (LSMeans) of or between the two treatment groups.

Safety: The safety data was to be summarized for all randomized subjects (intent-to-treat).

SUMMARY – CONCLUSIONS:

AMPICILLIN PHARMACOKINETIC RESULTS:

Of the 12 subjects completing the study, 11 were determined to be evaluable.

$AUC_{(0\text{-}inf)}$: The mean (SD) value for subjects administered pramlintide (90 µg) was 789.7 (191.1) µg*min/mL and the mean (SD) value for the same subjects administered placebo was 819.7 (252.4) µg*min/mL.

C_{max}: The mean (SD) values for the pramlintide (90 µg) and placebo treatments were 5.2 (1.0) µg/mL and 4.9 (1.7) µg/mL, respectively.

$t_{1/2}$: The mean (SD) for the pramlintide (90 µg) treatment was 50.7 (9.2) minutes and the mean (SD) for the placebo treatment was 59.4 (11.4) minutes.

T_{max} The mean (SD) ampicillin T_{max} value was 176.2 (36.6) minutes during the pramlintide (90 µg) treatment period and was 111.0 (44.0) minutes during the placebo treatment period.

The statistical analysis of the ampicillin results for the 11 evaluable subjects are presented in the following table.

PARAMETER	LSMEAN (SE)(1) {GEOMETRIC LSMEAN(2)} [95% C.I.(3)] A (4)	LSMEAN (SE)(1) {GEOMETRIC LSMEAN(2)} [95% C.I. (3)] B (4)	P-VALUE (1)	RATIO OR DIFFERENCE (5) OF THE LSMEANS	95% CONFIDENCE INTERVAL OF THE RATIO OR DIFFERENCE(5)
ln $AUC_{(0\text{-}8h)}$ (µg*min/mL)	6.7 (0.1) {782.3} [660.4, 926.8]	6.6 (0.1) {748.9} [632.2, 887.3]	0.553	95.7%	81.5%, 112.4%
ln $AUC_{(0\text{-}inf)}$ (µg*min/mL)	6.7 (0.1) {792.9} [667.7, 941.5]	6.6 (0.1) {759.3} [639.5, 901.7]	0.559	95.8%	81.5%, 112.5%
ln C_{max} (µg/mL)	1.5 (0.1) {4.7} [3.9, 5.6]	1.6 (0.1) {5.1} [4.3, 6.1]	0.380	108.3%	89.0%, 131.9%
$t_{(1/2)}$ (min)	59.1 (3.2) [52.4, 65.8]	51.0 (3.2) [44.2, 57.7]	0.046	-8.2	-16.2, -0.2
T_{max} (min)	110.0 (12.7) [83.3, 136.7]	176.2 (12.7) [149.5, 202.8]	0.003	66.2	29.1, 103.3

1 BASED ON AN ANALYSIS OF VARIANCE MODEL WHICH INCLUDED TERMS FOR SEQUENCE, SUBJECT-WITHIN-SEQUENCE, PERIOD AND TREATMENT

2 GEOMETRIC MEANS ARE THE ANTILOGS OF THE MEANS OF THE NATURAL LOGARITHMIC TRANSFORMED ENDPOINTS

3 FOR AUC AND C_{max}, THE C.I. IS BASED ON THE GEOMETRIC MEAN AND FOR T_{max} AND $t_{1/2}$, THE C.I. IS BASED ON THE RAW MEAN

4 A = PLACEBO AND B = PRAMLINTIDE

5 FOR THE GEOMETRIC LSMEANS THE RATIO = exp {ln[B] – ln[A]}*100 AND FOR THE UNTRANSFORMED LSMEANS THE DIFFERENCE = {B – A}

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events: No adverse events were reported during this study.

Deaths: No deaths were reported during this study.

Serious Adverse Events: No serious adverse events were reported during this study.

Clinical Laboratory Values: There were no laboratory parameters that appeared to be adversely affected by pramlintide or placebo.

Vital Signs and Physical Examination: No clinically significant values for heart rate and systolic and diastolic blood pressure were observed during the course of this study. At screening or study exit, ECG recordings and physical examination parameters were all normal.

CONCLUSIONS:

- A single 90 µg subcutaneous dose of pramlintide administered 15 minutes prior to a 500-mg oral dose of ampicillin does not alter the ampicillin pharmacokinetic parameters $AUC_{(0\text{-}inf)}$, C_{max} or $t_{1/2}$ in fasted, healthy, male and female subjects.

- Consistent with the effects of pramlintide on delaying the rate of nutrient delivery from the stomach, a single 90 µg subcutaneous dose of pramlintide administered 15 minutes prior to a 500-mg oral dose of ampicillin delays the ampicillin T_{max} from approximately 2 hours to approximately 3 hours, compared to placebo in fasted, healthy, male and female subjects.

- A single 90 µg subcutaneous dose of pramlintide administered 15 minutes prior to a 500-mg oral dose of ampicillin is generally well tolerated in fasted, healthy, male and female subjects.

Amylin Pharmaceuticals, Inc.
Pramlintide Protocol 137-130
[ ] Project 25046

1. PURPOSE

The original clinical study reports focused on the safety aspects of pramlintide treatment. In order to standardize the pharmacokinetic assessments across studies, a uniform analysis approach was applied in this addendum. While there are some specific differences between the analyses in this addendum and those in the clinical study report, the overall pharmacokinetic and pharmacodynamic findings and conclusions are generally similar.

The purpose of this addendum is 1) to provide additional analysis of glucose pharmacodynamics, 2) to compare the pharmacodynamic parameters using an ANCOVA model with the predose baseline glucose values as individual covariates, and 3) to provide mean and individual plots of baseline corrected plasma glucose.

2. SYNOPSIS

PK MEASURES AND METHODS: The pharmacodynamic effect on glucose following pramlintide administration was assessed by comparison of postprandial glucose concentrations following administration of pramlintide or placebo concomitantly with lispro insulin and NPH, lente, or ultralente insulin (administered as three separate subcutaneous injections). Parameters Cmax, AUC(0-300), Tmax, Cmin, and Tmin were compared between treatments. (For definitions of abbreviations and symbols, see Section 3).

RESULTS: Of the 12 Type 1 diabetic subjects initially dosed, data from the 11 subjects who completed at least one period were included in the pharmacodynamic and statistical analyses.

The arithmetic means and standard deviations of baseline corrected plasma glucose pharmacodynamic parameters and their statistical comparisons are summarized in the following table.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments A and B

	Plasma Glucose					
	Treatment B		Treatment A			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/dL)	84	75	124	86	18.9-109.3	64.1
Cmin(mg/dL)	-51	46	-45	68	224.4- 14.9	119.6
Tmax(min)	170	71	119	65	. - .	.
Tmin(min)	125	115	146	148	. - .	.
AUC(0-300)(mg*min/dL)	7205	14788	17055	24926	-39.5-114.9	37.7

Treatment B = 1 x 60 ug/0.1 mL SC Pramlintide Plus Lispro Insulin and NPH, Lente, or Ultralente Insulin (Test)
Treatment A = 1 x 0.1 mL SC Placebo Plus Lispro Insulin and NPH, Lente, or Ultralente Insulin (Reference)
* = Based on LS Means from Table 10.

CONCLUSIONS:

- Although individual responses were variable, based on the mean baseline corrected glucose concentration-time data, the overall differences in glucose pharmacodynamics were markedly different between the pramlintide and placebo treatment groups. A single 60 µg dose of pramlintide essentially neutralized the plasma glucose response following a meal.

- Compared to the placebo treatment, the pramlintide-treated group showed a mean reduction of 36% in Cmax and mean reduction of 62% in AUC(0-300) for baseline corrected glucose.

- Pramlintide (60 µg) delayed the time of onset for postprandial increases in glucose for approximately 90 min. In addition to decreasing the overall magnitude of the peak, pramlintide delayed the time to peak by close to 1 hr relative to placebo.

3. PHARMACODYNAMIC AND STATISTICAL MEASURES AND METHODS

To correlate with the methodology of other pramlintide protocols, actual times of blood sample collection for glucose measurement for this re-analysis were calculated with respect to drug (pramlintide or placebo) administration. Nominal times were still fixed at -10 (predose, baseline), 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, and 300 min but relative to study drug administration rather than to start of the meal. This introduced a difference of approximately 5 min in the blood draw time deviations compared with the data presented in the original report, since pramlintide and insulin injections were started and completed within 5 min prior to breakfast. The listing of the resultant blood draw times and deviations is presented in Appendix 2 to this addendum.

Baseline corrected glucose concentrations were obtained by subtracting the individual predose [baseline, t(0)] glucose concentration value from each of the subsequent glucose concentrations throughout the sampling period. The baseline used was specific to treatment. The resultant positive and negative concentrations were all included in the calculation of the parameters.

1. PURPOSE

The original clinical study reports focused on the safety aspects of pramlintide treatment. In order to standardize the pharmacokinetic assessments across studies, a uniform analysis approach was applied in this Addendum. While there are some specific differences between the analyses in this Addendum and those presented in the clinical study report, the overall pharmacokinetic and pharmacodynamic profiles and conclusions are generally similar.

The purpose of this addendum is 1) to provide additional analysis of pramlintide pharmacokinetics following each of the two treatments after deletion of concentration values below the quantifiable limit (BQL values), 2) to provide additional pharmacokinetic and statistical analyses for pramlintide and insulin using an ANCOVA model with the predose baseline values as individual covariates, 3) to provide additional pharmacodynamic and statistical analyses of glucose data following subtraction of baseline (predose) glucose levels, and 4) to provide mean and individual plots of pramlintide, insulin, and glucose concentrations versus time data.

2. SYNOPSIS

PK MEASURES AND METHODS:	Pharmacokinetic and pharmacodynamic effects of mixing pramlintide with insulin in the same syringe were assessed by measuring the concentrations of pramlintide, insulin, and glucose following co-administration of 30 µg pramlintide with Humulin® 70/30 as either single (Treatment B) or separate (Treatment A) SC injections. Equivalent bioavailability was shown if the 90% confidence intervals (CI) of the ratio of product means for Cmax, AUC(0-t), and AUC(0-inf) for pramlintide, and for Cmax, AUC(0-300), and AUC(0-600) for insulin were within the range of 80 to 125%. Pharmacodynamic effects on glucose were also assessed based by the comparison of Cmax, Tmax, Cmin, Tmin, and AUC(0-300) calculated from baseline-corrected glucose concentrations. (For definitions of abbreviations and symbols, see Section 3)
RESULTS:	Data from all 27 subjects enrolled in the study were included in the pharmacokinetic and statistical analyses.

The arithmetic means of plasma pramlintide pharmacokinetic parameters and their statistical comparisons are summarized in the following table.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments B and A

Pharmacokinetic Parameters	Plasma Pramlintide Treatment B Arithmetic Mean	SD	Treatment A Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	28.31	18.49	45.64	23.75	48.8- 75.6	62.2
Cmax/BMI(pmol/L)	1.21	0.89	1.92	1.17	48.7- 77.2	63.0
Tmax(min)	42.4	18.0	23.5	14.6	-	-
AUC(0-t)(pmol*hr/L)	2804	2241	3111	2288	74.7-106.6	90.7
AUC(0-t)/BMI(pmol*min/L)	120.5	108.2	133.5	112.6	73.6-108.1	90.8
AUC(0-inf)(pmol*min/L)	4741	2283	4536	2485	73.6-107.2	90.4
AUC(0-inf)/BMI(pmol*min/L)	205.1	118.4	193.0	127.4	70.8-108.3	89.6
T1/2(min)	73.1	34.3	59.6	35.4	98.6-145.4	122.0
Kel(1/min)	0.0109	0.00345	0.0144	0.00613	63.0- 96.2	79.6
AUCR	0.758	0.103	0.769	0.121	-	-
ln(Cmax)	3.221	0.5790	3.694	0.5153	51.0- 71.6	60.4
ln[AUC(0-t)]	7.635	1.050	7.806	0.7051	62.0-105.8	81.0
ln[AUC(0-inf)]	8.378	0.4113	8.280	0.5491	78.0-106.0	90.9

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Single SC Injection
Treatment A = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Separate SC Injections
* = Based on LS Means from Table 8.

The arithmetic means of serum insulin pharmacokinetic parameters and their statistical comparisons are summarized in the following table.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments B and A

Pharmacokinetic Parameters	Serum Insulin Treatment B Arithmetic Mean	SD	Treatment A Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	93.56	68.32	100.73	76.10	85.6-105.1	95.3
Cmin(min)	21.59	23.04	22.71	17.36	53.5-130.3	91.9
Tmax(min)	198	53.1	139	64.8	-	-
Tmin(min)	178	250	29.3	114	-	-
AUC(0-300)(uU*min/mL)	21021	16485	22870	16439	83.1-105.2	94.1
AUC(0-600)(uU*min/mL)	37085	29418	39378	29244	86.8-105.9	96.4
ln(Cmax)	4.350	0.5954	4.444	0.5356	81.9-105.8	93.1
ln(Cmin)	2.723	0.8223	2.909	0.6431	58.2-114.3	81.5
ln[AUC(0-300)]	9.754	0.5993	9.873	0.5364	80.7-102.7	91.0
ln[AUC(0-600)]	10.31	0.6176	10.40	0.5700	84.4-103.2	93.3

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Single SC Injection
Treatment A = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Separate SC Injections
* = Based on LS Means from Table 18.

The arithmetic means of baseline corrected plasma glucose pharmacodynamic parameters and their statistical comparisons are summarized in the following table.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments B and A

Pharmacokinetic Parameters	Plasma Glucose: Treatment B Arithmetic Mean	SD	Treatment A Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/dL)	80	75	69	71	86.0-144.9	115.5
Cmin(min)	-56	67	-74	78	110.8- 40.4	75.6
Tmax(min)	220	178	185	177	. - .	.
Tmin(min)	189	121	141	112	. - .	.
AUC(0-300)(mg*min/dL)	4057.2	17757	-2422.3	22284	86.3- -458	-185.9
AUC(0-600)(mg*min/dL)	8184.1	41932	-4927.6	46340	84.3- -439	-177.4

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Single SC Injection
Treatment A = 1 x 30 ug in 0.1 mL Pramlintide and Humulin 70/30 Insulin as Separate SC Injections
* = Based on LS Means from Table 29.

CONCLUSIONS:

- Mixing pramlintide with Humulin® 70/30 insulin in a single syringe resulted in an interaction between the two drugs which significantly affected the pharmacokinetics of pramlintide, particularly its rate of absorption. Following separate injections, pramlintide concentrations increased more rapidly and to a 40% greater Cmax and then declined with a 22% shorter half-life compared to mixed single injection.

- Mixing pramlintide with Humulin® 70/30 insulin in a single syringe had marginal effects on insulin bioavailability since, despite the longer median Tmax for Treatment B, the 90% CI for insulin Cmax, AUC(0-300), and AUC(0-600) for the comparison of Treatments B to A were within the acceptable 80% to 125% range. Based on the mean figures, serum-free insulin concentrations appeared to increase more quickly, then declined at a similar rate following separate injections of pramlintide and insulin compared with combined pramlintide and insulin.

- The combined effects on the pharmacokinetics of pramlintide and insulin following mixed administration resulted in baseline corrected plasma glucose concentration-time profiles that were consistently less for separate injections of pramlintide and insulin compared to mixed pramlintide and insulin.

- Statistical comparison of baseline corrected plasma glucose pharmacodynamic parameters showed that Treatment B had on average 15% greater Cmax and 24% greater Cmin compared to Treatment A, with no significant differences in Tmax or Tmin. However, the differences in the mean baseline corrected glucose AUC(0-300) values were dramatic, with a mean ratio of -186% for the comparison of Treatment B to A.

- While a pharmacokinetic interaction between pramlintide and Humulin 70/30 insulin significantly affects the pharmacokinetic parameters of pramlintide, suggesting that these two drugs should not be mixed in the same syringe prior to administration, there is no indication that mixing results in any undue risk to patients with regard to the insulin dose administered. The pharmacodynamic effects indicate potentially better glucose control with separate injections, however, contribution of the basal glucose values may make these differences not clinically significant.

3. PHARMACODYNAMIC AND STATISTICAL MEASURES AND METHODS

Although the limit of quantification (LOQ) for pramlintide for the IEMA assay is — pmol/L, due to dilution factors the actual limit of quantification for this study, referred to as BQL, was — pmol/L. Any value below that limit has been reset to zero in the concentration tables and for the purpose of pharmacokinetic analysis.

The following pharmacokinetic parameters were calculated from the pramlintide concentrations:

- Cmax — Maximum observed concentration within the sampling interval obtained without interpolation.
- Tmax — Time of the maximum observed concentration.
- AUC(0-t) — Area under the drug concentration-time curve from time zero to time t, AUC(0-t), where t is the time of the last measurable concentration (Ct).
- AUC(0-inf) — Area under the drug concentration-time curve from time zero to infinity, AUC(0-inf) = AUC(0-t) + Ct/Kel, where Kel is the terminal elimination rate constant.

Amylin Pharmaceuticals, Inc.
Pramlintide Protocol 137-119
[ ] Project 25042

1. PURPOSE

The original clinical study reports focused on the safety aspects of pramlintide treatment. In order to standardize the pharmacokinetic assessments across studies, a uniform analysis approach was applied in this addendum. While there are some specific differences between the analyses in this addendum and those in the clinical study report, the overall pharmacokinetic and pharmacodynamic findings and conclusions are generally similar.

The purpose of this addendum is 1) to provide additional analysis of pramlintide pharmacokinetics following each treatment after deletion of concentration values below the quantifiable limit (BQL values), 2) to provide additional pharmacokinetic and statistical analyses for pramlintide and insulin using an ANCOVA model with the predose baseline values as individual covariates, 3) to provide additional pharmacodynamic and statistical analyses of glucose data following subtraction of baseline (predose) glucose levels, and 4) to provide mean and individual plots of pramlintide, insulin, and glucose concentrations versus time data.

2. SYNOPSIS

The treatments were as follows:

Treatment A: 1 × 0.1 mL Placebo Plus NPH Plus Regular Insulin in One Syringe.

Treatment B: 1 × 30 µg/0.1 mL Pramlintide Plus NPH In One Syringe and Regular Insulin in a Separate Syringe.

Treatment C: 1 × 30 µg/0.1 mL Pramlintide Plus Regular Insulin in One Syringe and NPH in a Separate Syringe.

Treatment D: 1 × 30 µg/0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections.

Treatment E: 1 × 30 µg/0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe.

Comparisons of interest are summarized, by analyte, in the following table:

Pramlintide	Insulin	Glucose
B/D	A/D	E/A
C/D	B/D	E/D
E/D	C/D	B/D
	E/D	C/D
	A/E	

PK MEASURES AND METHODS:

Pharmacokinetic and pharmacodynamic effects of co-administering pramlintide with insulin mixed together in the same syringe in various combinations, as specified, and as separate subcutaneous injections were assessed by measuring the concentrations of pramlintide, insulin and glucose following each of the five treatments. Equivalent bioavailability of compared treatment pairs was shown for pramlintide and insulin if the 90% confidence intervals (CI) of the ratio of product means for Cmax, AUC(0-t), and AUC(0-inf) for pramlintide, and for Cmax, AUC(0-300), and AUC(0-600) for insulin were within the range of 80 to 125%. Pharmacodynamic effects on glucose were also assessed based by the comparison of Cmax, Tmax, Cmin, Tmin, and AUC(0-300) calculated from baseline-corrected glucose concentrations. (For definitions of abbreviations and symbols, see Section 3)

RESULTS:

Thirty-five subjects were randomized and the data from the 34 who completed at least one period were included in the analyses of glucose and insulin data. Subject 2021, whose data was not reportable due to assay interference, was excluded from the pharmacokinetic analysis of pramlintide, for a total of 33 subjects. Additionally, subject 1013, who completed only the placebo arm, was excluded from the statistical analyses for pramlintide for a total of 32 subjects.

The arithmetic means of plasma pramlintide pharmacokinetic parameters and their statistical comparisons for Treatments B, C, and E versus Treatment D are summarized in the following 3 tables.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments B and D

	Plasma Pramlintide					
	Treatment B		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	37.87	16.45	42.20	20.11	80.3- 97.3	88.8
Cmax/BMI(pmol/L)	1.65	0.87	1.86	1.04	79.0- 96.1	87.6
Tmax(min)	27.9	11.2	17.3	5.23	. - .	.
AUC(0-t)(pmol*hr/L)	2730	1641	2309	1569	97.2-130.0	113.6
AUC(0-t)/BMI(pmol*min/L)	119.4	82.22	103.5	78.72	91.8-127.7	109.8
AUC(0-inf)(pmol*min/L)	3778	1749	3088	1694	97.4-126.6	112.0
AUC(0-inf)/BMI(pmol*min/L)	166.0	90.24	137.1	87.45	93.1-123.9	108.5
T1/2(min)	49.7	18.3	37.5	14.6	105.8-131.1	118.4
Kel(1/min)	0.0153	0.00425	0.0211	0.00771	67.1- 87.4	77.2
AUCR	0.777	0.0874	0.794	0.103	. - .	.
ln(Cmax)	3.539	0.4565	3.625	0.5150	82.0-102.8	91.8
ln[AUC(0-t)]	7.718	0.6805	7.502	0.7719	104.0-146.9	123.6
ln[AUC(0-inf)]	8.141	0.4450	7.914	0.4915	102.7-134.3	117.4

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus NPH in One Syringe and Regular Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 19.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments C and D

Pharmacokinetic Parameters	Plasma Pramlintide Treatment C Arithmetic Mean	SD	Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	37.13	16.30	42.20	20.11	76.8- 93.9	85.3
Cmax/BMI(pmol/L)	1.63	0.87	1.86	1.04	76.4- 93.4	84.9
Tmax(min)	22.6	11.6	17.3	5.23	. - .	.
AUC(0-t)(pmol*hr/L)	2355	1742	2309	1569	79.1-111.9	95.5
AUC(0-t)/BMI(pmol*min/L)	105.5	88.19	103.5	78.72	77.1-113.1	95.1
AUC(0-inf)(pmol*min/L)	3262	1902	3088	1694	83.1-112.2	97.7
AUC(0-inf)/BMI(pmol*min/L)	146.1	99.68	137.1	87.45	81.5-112.3	96.9
T1/2(min)	47.1	20.7	37.5	14.6	106.0-131.3	118.6
Kel(1/min)	0.0171	0.00625	0.0211	0.00771	70.2- 90.4	80.3
AUCR	0.768	0.0862	0.794	0.103	. - .	.
ln(Cmax)	3.508	0.4979	3.625	0.5150	77.2- 96.8	86.5
ln[AUC(0-t)]	7.495	0.7902	7.502	0.7719	79.8-112.7	94.8
ln[AUC(0-inf)]	7.939	0.5607	7.914	0.4915	86.9-113.7	99.4

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Regular Insulin in One Syringe and NPH in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 20.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments E and D

Pharmacokinetic Parameters	Plasma Pramlintide Treatment E Arithmetic Mean	SD	Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	36.18	13.93	42.20	20.11	78.6- 95.6	87.1
Cmax/BMI(pmol/L)	1.57	0.77	1.86	1.04	78.4- 95.4	86.9
Tmax(min)	32.6	11.7	17.3	5.23	. - .	.
AUC(0-t)(pmol*hr/L)	3048	1880	2309	1569	114.0-146.9	130.4
AUC(0-t)/BMI(pmol*min/L)	135.0	102.5	103.5	78.72	112.6-148.5	130.5
AUC(0-inf)(pmol*min/L)	4320	1762	3088	1694	113.9-143.0	128.5
AUC(0-inf)/BMI(pmol*min/L)	169.9	103.3	137.1	87.45	111.8-142.6	127.2
T1/2(min)	54.8	13.0	37.5	14.6	123.4-148.6	136.0
Kel(1/min)	0.0134	0.00323	0.0211	0.00771	56.3- 76.6	66.4
AUCR	0.772	0.0916	0.794	0.103	. - .	.
ln(Cmax)	3.503	0.4469	3.625	0.5150	79.3- 99.5	88.8
ln[AUC(0-t)]	7.821	0.6979	7.502	0.7719	113.7-160.7	135.2
ln[AUC(0-inf)]	8.298	0.3863	7.914	0.4915	119.0-155.7	136.1

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 21.

The arithmetic means of serum insulin pharmacokinetic parameters and their statistical comparisons for Treatments A, B, C, and E versus Treatment D are summarized in the following 4 tables.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments A and D

	Serum Insulin					
	Treatment A		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	122.58	109.30	121.41	116.31	78.5-130.1	104.3
Cmin(min)	30.33	35.01	38.98	51.88	58.1-101.3	79.7
Tmax(min)	193	59.6	144	83.6	. - .	.
Tmin(min)	191	258	337	273	. - .	.
AUC(0-300)(uU*min/mL)	27031	24657	27640	26633	85.4-117.7	101.6
AUC(0-600)(uU*min/mL)	46539	50045	46076	50957	93.1-120.9	107.0
ln(Cmax)	4.612	0.5728	4.566	0.6158	90.0-125.5	106.3
ln(Cmin)	3.099	0.7568	3.288	0.8053	62.7-100.9	79.5
ln[AUC(0-300)]	10.011	0.55497	9.9955	0.61471	91.6-116.8	103.4
ln[AUC(0-600)]	10.523	0.57588	10.480	0.62219	95.9-120.4	107.5

Treatment A = 1 x 0.1 mL Placebo Plus NPH Plus Regular Insulin in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 48.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments B and D

	Serum Insulin					
	Treatment B		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	126.85	99.84	121.41	116.31	79.2-134.2	106.7
Cmin(min)	35.16	33.19	38.98	51.88	68.3-114.4	91.3
Tmax(min)	158	60.5	144	83.6	. - .	.
Tmin(min)	388	264	337	273	. - .	.
AUC(0-300)(uU*min/mL)	29981	25130	27640	26633	94.3-128.7	111.5
AUC(0-600)(uU*min/mL)	48179	42667	46076	50957	93.9-123.6	108.8
ln(Cmax)	4.675	0.5304	4.566	0.6158	93.8-133.6	111.9
ln(Cmin)	3.243	0.7849	3.288	0.8053	70.1-116.5	90.3
ln[AUC(0-300)]	10.127	0.54443	9.9955	0.61471	100.1-129.6	113.9
ln[AUC(0-600)]	10.588	0.55735	10.480	0.62219	99.9-127.3	112.8

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus NPH in One Syringe and Regular Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 49.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments C and D

Pharmacokinetic Parameters	Serum Insulin Treatment C Arithmetic Mean	SD	Serum Insulin Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	132.65	82.49	121.41	116.31	80.3-134.3	107.3
Cmin(min)	36.94	34.42	38.98	51.88	72.3-117.5	94.9
Tmax(min)	144	74.0	144	83.6	. - .	.
Tmin(min)	395	241	337	273	. - .	.
AUC(0-300)(uU*min/mL)	26492	18074	27640	26633	78.1-111.9	95.0
AUC(0-600)(uU*min/mL)	42646	31729	46076	50957	78.5-107.7	93.1
ln(Cmax)	4.722	0.5802	4.566	0.6158	93.9-133.0	111.8
ln(Cmin)	3.354	0.6880	3.288	0.8053	78.1-128.7	100.3
ln[AUC(0-300)]	10.037	0.51749	9.9955	0.61471	88.7-114.3	100.7
ln[AUC(0-600)]	10.499	0.53277	10.480	0.62219	89.0-112.9	100.3

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Regular Insulin in One Syringe and NPH in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table S0.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments E and D

Pharmacokinetic Parameters	Serum Insulin Treatment E Arithmetic Mean	SD	Serum Insulin Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	134.01	159.80	121.41	116.31	102.4-158.1	130.2
Cmin(min)	29.72	30.42	38.98	51.88	60.0-106.7	83.4
Tmax(min)	203	109	144	83.6	. - .	.
Tmin(min)	218	271	337	273	. - .	.
AUC(0-300)(uU*min/mL)	26840	31511	27640	26633	94.7-129.6	112.2
AUC(0-600)(uU*min/mL)	44305	49715	46076	50957	95.1-125.2	110.2
ln(Cmax)	4.544	0.7718	4.566	0.6158	91.7-131.4	109.8
ln(Cmin)	3.115	0.6936	3.288	0.8053	68.0-113.8	88.0
ln[AUC(0-300)]	9.8930	0.71061	9.9955	0.61471	87.8-114.1	100.1
ln[AUC(0-600)]	10.425	0.67030	10.480	0.62219	92.1-117.7	104.1

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table S1.

The arithmetic means of serum insulin pharmacokinetic parameters and their statistical comparisons for Treatment A versus Treatment E is summarized in the following table

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments A and E

Pharmacokinetic Parameters	Serum Insulin Treatment A Arithmetic Mean	SD	Serum Insulin Treatment E Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	122.58	109.30	134.01	159.80	60.1-100.0	80.1
Cmin(min)	30.33	35.01	29.72	30.42	69.5-121.7	95.6
Tmax(min)	193	59.6	203	109	. - .	.
Tmin(min)	191	258	218	271	. - .	.
AUC(0-300)(uU*min/mL)	27031	24657	26840	31511	76.1-105.0	90.5
AUC(0-600)(uU*min/mL)	46539	50045	44305	49715	84.4-109.9	97.1
ln(Cmax)	4.612	0.5728	4.544	0.7718	81.9-114.5	96.8
ln(Cmin)	3.099	0.7568	3.115	0.6936	71.1-114.9	90.4
ln[AUC(0-300)]	10.011	0.55497	9.8930	0.71061	91.5-116.8	103.3
ln[AUC(0-600)]	10.523	0.57588	10.425	0.67030	92.0-115.7	103.2

Treatment A = 1 x 0.1 mL Placebo Plus NPH Plus Regular Insulin in One Syringe
Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe
* = Based on LS Means from Table 52.

The arithmetic means of baseline corrected plasma glucose pharmacodynamic parameters and their statistical comparisons for Treatments E versus A and Treatments E, B and C versus D are summarized in the following 4 tables.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments E and A

Pharmacokinetic Parameters	Plasma Glucose Treatment E Arithmetic Mean	SD	Plasma Glucose Treatment A Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	80	74	128	74	49.0- 81.6	65.3
Cmin(min)	-106	99	-125	112	110.4- 63.7	87.1
Tmax(min)	148	148	204	190	. - .	.
Tmin(min)	266	217	321	209	. - .	.
AUC(0-300)(mg*min/mL)	-2072.1	25584	7567.2	23071	-65.9- 67.4	0.7
AUC(0-600)(mg*min/mL)	-5071.4	50659	-74.547	47528	-405-291.9	-56.7

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe
Treatment A = 1 x 0.1 mL Placebo Plus NPH Plus Regular Insulin in One Syringe
* = Based on LS Means from Table 79.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments E and D

	Plasma Glucose					
	Treatment E		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	80	74	109	100	56.0- 93.5	74.7
Cmin(min)	-106	99	-103	109	136.4- 78.3	107.4
Tmax(min)	148	148	264	219	. - .	.
Tmin(min)	266	217	233	175	. - .	.
AUC(0-300)(mg*min/mL)	-2072.1	25584	-2403.0	30603	-804-821.5	8.8
AUC(0-600)(mg*min/mL)	-5071.4	50659	2777.5	65026	-212-152.8	-29.4

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, NPH Plus Regular Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 80.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments B and D

	Plasma Glucose					
	Treatment B		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	67	63	109	100	45.9- 83.7	64.8
Cmin(min)	-124	94	-103	109	149.9- 91.4	120.6
Tmax(min)	225	221	264	219	. - .	.
Tmin(min)	265	203	233	175	. - .	.
AUC(0-300)(mg*min/mL)	-8411.6	20525	-2403.0	30603	-1409-226.6	-591.4
AUC(0-600)(mg*min/mL)	-13778	45693	2777.5	65026	-310- 56.5	-126.8

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus NPH in One Syringe and Regular Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 81.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments C and D

	Plasma Glucose					
	Treatment C		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	88	75	109	100	63.0-100.7	81.9
Cmin(min)	-119	97	-103	109	146.3- 88.1	117.2
Tmax(min)	252	222	264	219	. - .	.
Tmin(min)	264	199	233	175	. - .	.
AUC(0-300)(mg*min/mL)	-4798.8	25264	-2403.0	30603	-969-659.7	-154.7
AUC(0-600)(mg*min/mL)	-3016.7	53740	2777.5	65026	-162-203.0	20.5

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Regular Insulin in One Syringe and NPH in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Regular Insulin and NPH Each as Separate Injections
* = Based on LS Means from Table 82.

CONCLUSIONS:

- Mixing pramlintide with NPH and/or regular insulin in a single syringe substantially affected the pharmacokinetics of pramlintide, as none of the mixed Treatments B, C, or E could be considered bioequivalent to unmixed Treatment D. Mixing pramlintide with regular insulin decreased Cmax by approximately 12%, while mixing pramlintide with NPH nearly doubled the median Tmax and increased the extent of pramlintide absorption. Mixing of all three compounds combined these effects.

- Comparison of the placebo mixed with the two insulin types in the same syringe (Treatment A) with Treatment D (pramlintide, regular insulin, and NPH in separate syringes), and with Treatment E (pramlintide, regular insulin, and NPH mixed in the same syringe) indicates relatively equivalent rate and extent of insulin absorption in the presence of pramlintide compared to the placebo, regardless of whether the pramlintide is given in a separate syringe or mixed.

- Mixing pramlintide with NPH and/or regular insulin in a single syringe had variable effects on insulin pharmacokinetics. Neither Treatments B, C, nor E could be considered bioequivalent to Treatment D. The rate and/or extent of insulin absorption was variable with the magnitude and direction of change depending on whether NPH or regular insulin were mixed with pramlintide.

- Comparison of mean baseline corrected concentration time profiles for glucose indicates that addition of pramlintide to insulin, even if the two insulin types and pramlintide were mixed in the same syringe (Treatment E), essentially abolished the effect of food by maintaining glucose concentrations at or below baseline levels through at least the first 300 min after the meal compared to when the two insulins were given alone (Treatment A). Based on the mean figures, administering pramlintide in a separate syringe from the two insulin types (Treatment D) further decreased the baseline corrected glucose concentrations, at least within the first 180 min after meal, compared to when pramlintide, NPH and regular insulin were given in a single injection (Treatment E).

- Comparisons of baseline corrected data from Treatments B and C versus Treatment D showed that mixing pramlintide with one of the insulins also reduced the mean glucose Cmax and AUC(0-300) compared to when pramlintide and both insulins were given as three separate injections. The effect of mixing NPH with pramlintide (Treatment B) was more exaggerated compared to when regular insulin was mixed with pramlintide (Treatment C).

- Because of the variable and inconsistent effect of mixing on pramlintide and insulin concentrations, it is recommended that NPH and/or regular insulin should not be mixed with pramlintide, however, there is no indication that mixing results in any undue risk to patients with regard to glucose control.

3. PHARMACODYNAMIC AND STATISTICAL MEASURES AND METHODS

Although the lower limit of quantitation (LLOQ) for pramlintide for the IEMA assay is — pmol/L, due to the lot of standards used, the actual limit of quantitation for this study, referred to as BQL, was — pmol/L. Any value below that limit has been reset to zero in the concentration tables and for the purpose of pharmacokinetic analysis.

The following pharmacokinetic parameters were calculated from the pramlintide concentrations:

- Cmax — Maximum observed concentration within the sampling interval obtained without interpolation.
- Tmax — Time of the maximum observed concentration.
- AUC(0-t) — Area under the drug concentration-time curve from time zero to time t, AUC(0-t), where t is the time of the last measurable concentration (Ct).
- AUC(0-inf) — Area under the drug concentration-time curve from time zero to infinity, AUC(0-inf) = AUC(0-t) + Ct/Kel, where Kel is the terminal elimination rate constant.
- AUCR — The ratio of AUC(0-t) to AUC(0-inf).
- T1/2 — Effective elimination half-life, calculated as ln(2)/Kel.
- Kel — Apparent first-order elimination or terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-squares regression analysis using the last three (or more) non-zero plasma concentrations.

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)
Protocol No.: 137-145		
Title of Study: An Open-Label, Randomized, Two-Period Crossover Study in Healthy Volunteers to Test the Bioequivalence of Pramlintide Supplied by Two Different Manufacturers		
Investigators and Study Centers: [ ]		
Publication (Reference): None		
Studied Period (Years): November 2000		**Phase of Development:** 1
Objective: To establish the bioequivalence of single subcutaneous (SC) injections of a formulated pramlintide product, with the active ingredient supplied by two different manufacturers.		
Methodology: This was a single-center, open-label, randomized, two-sequence crossover study consisting of two treatment periods (Period 1 and Period 2), with a 24-hour interval between each period. Subjects received the following single dose treatments (A and B) of pramlintide on alternating days as scheduled according to a balanced, randomization scheme. A. 60 µg in a 1.0 mg/mL formulation in cartridge form with active ingredient manufactured by [ ] B. 60 µg in a 1.0 mg/mL formulation in cartridge form with active ingredient manufactured by [ ]		
Number of Subjects: Thirty subjects were enrolled in this study. Evaluable subjects were defined as subjects who satisfied all study entry criteria, completed both treatment periods, and had adequate plasma pramlintide concentration data to allow reliable calculation of pharmacokinetic parameters.		
Key Demographics: Of the 30 randomized subjects, 16 (53.3%) were female and 14 (46.7%) were male. The majority of subjects were Hispanic (90.0%), and the mean age of the subject population was 38.4 years. Age, height, weight, and body mass index (BMI) appeared to be similar between treatment sequences.		
Subject Disposition: All 30 subjects completed all study procedures and study visits (screening, baseline [Day 0], Period 1 [Day 1], Period 2 [Day 2], and study exit [Day 2]).		
Diagnosis and Main Criteria for Inclusion: Normal, healthy males or females, aged between 18 and 65 years, who met all other protocol defined inclusion and exclusion criteria.		
Test Form, Dose and Mode of Administration, Batch No.: [ ] pramlintide, 1.0 mg/mL, 0.06 mL per subject, pen administration of SC injection, Lot number: (AC137-F28) 99-0603KB.		
Duration of Treatment: Two single-dose periods with at least a 24-hour interval between doses.		
Reference Form, Dose and Mode of Administration, Batch No: [ ] pramlintide, 1.0 mg/mL, 0.06 mL per subject, pen administration of SC injection, Lot number: (AC137-F28) 99-0602KB.		

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: pramlintide injection	Volume:	
NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	Page:	

Criteria for Evaluation:

Pharmacokinetics: The primary pharmacokinetic measures for demonstrating bioequivalence were area under the plasma concentration-time curve from time zero to last time point ($AUC_{(0\text{-}300\ min)}$) and peak concentration (C_{max}). Other pharmacokinetic measures included time to peak concentration (T_{max}), terminal half-life ($t_{1/2}$), and area under the plasma-concentration-time curve from time zero to infinity ($AUC_{(0\text{-}\infty)}$).

Safety: Safety and tolerability were assessed throughout the study period by monitoring adverse events, clinical laboratory measures, vital signs, physical examinations, and collecting anti-pramlintide antibody data.

Statistical Methods:

Pharmacokinetics: Bioequivalence between the two treatments [redacted] was based on an ANOVA of natural log transformed $AUC_{(0\text{-}300\ min)}$ and C_{max} parameters. Ninety percent confidence intervals were calculated for the ratios of the least squares (LS) geometric means between the pramlintide active ingredient supplied by [redacted] Bioequivalence was concluded if the 90% confidence intervals fell within 80% to 125%.

Safety: Adverse events, clinical laboratory measures, and vital signs were presented using descriptive statistics.

SUMMARY - CONCLUSIONS:

PHARMACOKINETIC RESULTS: Pramlintide from two manufacturers [redacted] was shown to be bioequivalent when administered SC using a pen-cartridge delivery system: the 90% confidence intervals for the ratios of the LS geometric means for C_{max}, $AUC_{(0\text{-}300\ min)}$, and $AUC_{(0\text{-}\infty)}$ all fell within 80% to 125%. In addition, the mean T_{max} and $t_{1/2}$ remained relatively unchanged.

Plasma Pramlintide Pharmacokinetic Parameters: Statistical Analysis From An ANOVA Model

Parameter (Unit)	Treatment A (mean)* N=29	Treatment B (mean)* N=29	Ratio (A/B) or Difference of (A-B)†	90% C.I of the Ratio or 95% C.I. of the Difference†	Treatment p-value
C_{max} (pmol/L)	50.4 (2.99)	51.8 (3.08)	0.97	(0.894, 1.059)	0.5817
$AUC_{(0\text{-}300\ min)}$ (pmol•min/L)	2463.1 (247.41)	2554.4 (256.58)	0.96	(0.856, 1.086)	0.6067
$AUC_{(0\text{-}\infty)}$ (pmol•min/L)	3631.6 (316.99)	3609.0 (312.26)	1.01	(0.896, 1.131)	0.9279
T_{max} (min)	17.8 (1.91)	16.2 (1.91)	1.63	(-2.981, 6.238)	0.4747
$t_{1/2}$ (min)	55.8 (6.45)	56.9 (6.37)	-1.11	(-14.475, 12.254)	0.8657

Note: Treatment A: 60 µg, 1.0 mg/mL formulation in cartridge form from [redacted] (Lot No: 99-0603KB).
Treatment B: 60 µg, 1.0 mg/mL formulation in cartridge form from [redacted] (Lot No: 99-0602KB).

*LS geometric means (anti-log of the LS means of logs) for natural log-transformed parameters, arithmetic means for T_{max} and $t_{1/2}$.
†Ratio (A/B) and 90% C.I. for C_{max}, $AUC_{(0\text{-}300\ min)}$, and $AUC_{(0\text{-}\infty)}$, difference (A-B) and 95% C.I. for T_{max} and $t_{1/2}$.

SYNOPSIS (continued)

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

SAFETY RESULTS:

Adverse Events: No treatment-emergent adverse events were reported during this study.

Clinical Laboratory Values: No clinically meaningful changes were reported for hematology or blood chemistry values. Five subjects (107, 112, 121, 123, and 128) had clinically noteworthy elevations in urine glucose concentrations at study exit (Day 2) that were not present at baseline (Day 0). Three of these subjects returned after study end for unscheduled glucose tolerance tests (GTT) to further evaluate these observations. Although these subjects met the inclusion criteria of healthy subjects at screening, these subjects evidenced high postprandial glucose concentrations during the oral GTTs indicating abnormal glucose tolerance. After careful review of all available laboratory data, the Investigator concluded that there was no relationship between the incidence of glucosuria and study medication.

Vital Signs and Physical Examinations: No clinically meaningful or unexpected changes were reported in vital signs or physical examinations.

Anti-Pramlintide Antibodies: All subjects had a negative anti-pramlintide antibody response at study exit (Day 2).

CONCLUSIONS:

Pramlintide from two manufacturers [ ] was shown to be bioequivalent when administered SC using a pen-cartridge delivery system.

Pramlintide from two manufacturers [ ] was well-tolerated when administered SC using a pen-cartridge delivery system.

Date of the report: 07 March 2001

Amylin Pharmaceuticals
Pramlintide Protocol 137-143
[ ] Project 24958

REPORT SYNOPSIS

TITLE:	An Open-Label Assessment of the Single Dose and Multiple Dose Pharmacokinetic Profiles of Pramlintide in Subjects With Type 1 Diabetes Mellitus
SPONSOR:	Amylin Pharmaceuticals, Inc. 9373 Towne Centre Drive San Diego, CA 92121
STUDY SITE:	[ ]
PRINCIPAL INVESTIGATOR:	[ ]
OBJECTIVES:	The primary objective of this study was to assess the single dose and multiple dose pharmacokinetic profiles of subcutaneously (SC) administered pramlintide in subjects with type 1 diabetes mellitus. The secondary objective was to assess the single dose and multiple dose pharmacodynamic profiles of glucose in subjects with type 1 diabetes mellitus treated with pramlintide.
STUDY DESIGN:	This study had a randomized, open-label, three-treatment, three-way crossover design with two dosing frequency groups: TID and QID.

TREATMENTS: A-F: Pramlintide 1.0 mg/mL
(1.5 mL multiple dose cartridges)
Manufactured by [ ]
Lot No.: 97-0403KB
Expiration date: 10/17/2000

Subjects in Group 1 were assigned to the TID pramlintide treatments A-C in a randomized fashion, as follows:

Group 1: Treatment A: 30 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses).

Treatment B: 60 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses).

Treatment C: 90 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses).

Subjects in Group 2 were assigned to the QID pramlintide treatments D-F in a randomized fashion, as follows:

Group 2: Treatment D: 30 µg, SC, QID, given just prior to breakfast, lunch, dinner, and evening snack for 4 days followed by a single dose prior to breakfast on the 5th day (17 consecutive doses).

Treatment E: 60 µg, SC, QID, given just prior to breakfast, lunch, dinner, and evening snack for 4 days followed by a single dose prior to breakfast on the 5th day (17 consecutive doses).

Treatment F: 90 µg, SC, QID, given just prior to breakfast, lunch, dinner, and evening snack for 4 days

followed by a single dose prior to breakfast on the 5th day (17 consecutive doses).

PK MEASURES AND METHODS:

Pramlintide doses were administered with [ ] Insulin Pen and the cartridge device intended for marketing. Pharmacokinetic (pramlintide) and pharmacodynamic (glucose) assessments were made following administration of the first and last doses of each of the six subcutaneous pramlintide dosing regimens by measuring serial plasma concentrations from which the parameters C_{max}, T_{max}, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, AUCR, $t_{½}$, and Kel were calculated for pramlintide, and parameters C_{max}, T_{max}, C_{min}, T_{min}, and $AUC_{(0-300)}$ were calculated for baseline corrected glucose. At each dose level, subjective comparisons were performed between Day 1 and Day 5 parameters for the same dose level. Subjective dose proportionality assessment was performed for pramlintide C_{max} and $AUC_{(0-t)}$ for parameters of subjects within each group.

RESULTS:

For the TID group, 11 subjects completed Treatments A, B, and C, Day 1; 11 subjects completed Treatment B, Day 5; and 10 subjects completed Treatments A and C, Day 5. For the QID group, 11 subjects completed Treatment D (Days 1 and 5); 10 subjects completed Treatment E (Days 1 and 5); and 12 subjects completed Day 1 and 11 subjects completed Day 5 of Treatment F. Data from all subjects who completed at least one dosing period were included in the pharmacokinetic and pharmacodynamic analyses.

Arithmetic means of the plasma pramlintide pharmacokinetic parameters for all 6 treatments are summarized in the following four tables.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

Pharmacokinetic Parameters	Plasma Pramlintide Treatment A N=11 Arithmetic Mean	SD	Treatment B N=11 Arithmetic Mean	SD	Treatment C N=11 Arithmetic Mean	SD
Cmax(pmol/L)	41.9	22.9	64.5	23.7	99.4	31.4
Tmax(hr)	0.273	0.0753	0.365	0.237	0.321	0.115
AUC(0-t)(pmol*hr/L)	32.86	28.40	74.14	34.26	118.5	65.37
AUC(0-inf)(pmol*hr/L)	81.37	22.17	102.6	37.24	144.2	65.74
T1/2(hr)	1.12	1.01	0.970	0.337	0.774	0.281
Kel(1/hr)	0.887	0.374	0.796	0.278	1.03	0.423
AUCR	0.738	0.155	0.784	0.0650	0.879	0.0308

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

Pharmacokinetic Parameters	Plasma Pramlintide Treatment A N=10 Arithmetic Mean	SD	Treatment B N=11 Arithmetic Mean	SD	Treatment C N=10 Arithmetic Mean	SD
Cmax(pmol/L)	37.6	22.8	74.4	20.0	92.7	26.7
Tmax(hr)	0.328	0.120	0.334	0.113	0.288	0.0827
AUC(0-t)(pmol*hr/L)	26.45	21.93	79.68	48.98	104.8	63.53
AUC(0-inf)(pmol*hr/L)	64.96	9.629	117.2	44.39	136.3	66.70
T1/2(hr)	0.726	0.239	0.789	0.326	0.722	0.230
Kel(1/hr)	1.02	0.282	1.00	0.384	1.03	0.281
AUCR	0.800	0.0873	0.835	0.0707	0.867	0.0455

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

	Plasma Pramlintide					
	Treatment D N=11		Treatment E N=10		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(pmol/L)	36.5	10.2	66.9	25.0	123.9	40.5
Tmax(hr)	0.274	0.0751	0.276	0.0794	0.273	0.0721
AUC(0-t)(pmol*hr/L)	20.10	11.46	51.67	33.37	115.9	54.29
AUC(0-inf)(pmol*hr/L)	48.88	.	72.01	37.82	129.1	55.74
T1/2(hr)	0.595	.	0.656	0.169	0.645	0.165
Kel(1/hr)	1.16	.	1.13	0.316	1.14	0.284
AUCR	0.801	.	0.806	0.0897	0.883	0.0544

Treatment D = 1 x 30 ug Pramlintide QID
Treatment E = 1 x 60 ug Pramlintide QID
Treatment F = 1 x 90 ug Pramlintide QID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

	Plasma Pramlintide					
	Treatment D N=11		Treatment E N=10		Treatment F N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(pmol/L)	40.7	20.0	70.7	23.3	98.6	30.1
Tmax(hr)	0.249	0.00205	0.278	0.0797	0.275	0.0818
AUC(0-t)(pmol*hr/L)	24.20	18.76	63.88	49.83	102.0	41.77
AUC(0-inf)(pmol*hr/L)	66.43	15.99	98.34	56.22	124.1	46.12
T1/2(hr)	0.713	0.173	0.724	0.303	1.02	1.02
Kel(1/hr)	1.00	0.243	1.07	0.342	1.03	0.496
AUCR	0.745	0.0730	0.852	0.0614	0.828	0.129

Treatment D = 1 x 30 µg Pramlintide QID
Treatment E = 1 x 60 µg Pramlintide QID
Treatment F = 1 x 90 µg Pramlintide QID

The arithmetic means of the baseline corrected plasma glucose pharmacodynamic parameters for all six treatments are summarized in the following four tables. Mean C_{max} values reflect the maximum magnitude of glucose increase following meal, while mean C_{min} values reflect the maximum glucose decrease.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 1

	Baseline Corrected Plasma Glucose					
	Treatment A N=11		Treatment B N=11		Treatment C N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	77.64	71.98	54.34	61.13	39.40	45.84
Cmin(mg/dL)	-38.30	37.81	-47.47	63.24	-68.35	79.40
Tmax(hr)	2.53	1.88	2.40	1.90	2.17	2.16
Tmin(hr)	2.39	1.66	1.58	1.18	2.21	1.68
AUC(0-300)(mg*hr/dL)	69.92	278.2	5.804	268.9	-88.89	294.0

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 5

	Baseline Corrected Plasma Glucose					
	Treatment A N=10		Treatment B N=11		Treatment C N=10	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	47.60	48.15	37.09	58.07	44.42	44.00
Cmin(mg/dL)	-87.90	93.56	-105.1	72.02	-49.64	59.42
Tmax(hr)	1.85	1.68	1.49	1.83	2.90	2.07
Tmin(hr)	3.15	1.84	3.41	1.73	1.75	1.57
AUC(0-300)(mg*hr/dL)	-97.17	367.0	-188.3	356.1	-29.03	301.6

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 1

	Baseline Corrected Plasma Glucose					
	Treatment D N=11		Treatment E N=10		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	71.19	69.92	50.80	62.83	35.50	37.51
Cmin(mg/dL)	-83.85	95.00	-91.03	98.90	-80.16	58.98
Tmax(hr)	1.99	1.75	2.03	1.97	1.95	1.82
Tmin(hr)	2.70	2.02	3.55	1.87	3.09	1.87
AUC(0-300)(mg*hr/dL)	-42.84	368.9	-48.43	357.2	-100.1	213.3

Treatment D = 1 x 30 µg Pramlintide QID
Treatment E = 1 x 60 µg Pramlintide QID
Treatment F = 1 x 90 µg Pramlintide QID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 5

	Baseline Corrected Plasma Glucose					
	Treatment D N=11		Treatment E N=10		Treatment F N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	72.26	68.40	48.30	53.65	67.36	66.73
Cmin(mg/dL)	-90.79	114.86	-104.7	109.67	-87.57	57.42
Tmax(hr)	2.23	1.92	2.30	1.83	2.96	2.12
Tmin(hr)	2.59	2.01	2.75	1.60	2.64	1.94
AUC(0-300)(mg*hr/dL)	-33.61	482.9	-192.1	505.4	-89.70	293.4

Treatment D = 1 x 30 µg Pramlintide QID
Treatment E = 1 x 60 µg Pramlintide QID
Treatment F = 1 x 90 µg Pramlintide QID

CONCLUSION:

- Pramlintide did not accumulate in the plasma of subjects with type 1 diabetes during the 5-day SC administration of either 30 µg, 60 µg, or 90 µg, TID or QID.

- Mean pramlintide concentration pharmacokinetic parameters C_{max} and $AUC_{(0-t)}$ increased relatively proportionally with increasing doses of pramlintide across the 30 to 90 µg dosing range.

- Baseline corrected glucose concentrations generally remained in a range similar to or less than predose, pre-meal levels throughout the 5-hour post-breakfast period in these subjects with type 1 diabetes. This was observed on both Day 1 and Day 5. The lack of a placebo group and the presence of confounding factors including insulin dosage timing relative to the meal, amount of meal consumed, and an incomplete record of insulin usage during the study periods make it difficult to draw pharmacodynamic conclusions.

REPORT SYNOPSIS

TITLE:	An Open-Label Assessment of the Single Dose and Multiple Dose Pharmacokinetic Profiles of Pramlintide in Subjects With Type 2 Diabetes Mellitus
SPONSOR:	Amylin Pharmaceuticals, Inc. 9373 Towne Centre Drive San Diego, CA 92121
STUDY SITE:	[ ]
PRINCIPAL INVESTIGATOR:	[ ]
OBJECTIVES:	The primary objective of this study was to assess the single dose and multiple dose pharmacokinetic profiles of subcutaneously (SC) administered pramlintide in subjects with type 2 diabetes mellitus. The secondary objective was to assess the single dose and multiple dose pharmacodynamic profiles of glucose in subjects with type 2 diabetes mellitus treated with pramlintide.
STUDY DESIGN:	This study had a randomized, open-label, three-treatment, three-way crossover design with two dosing frequency groups: BID and TID.

TREATMENTS: A-F: Pramlintide 1.0 mg/mL
(1.5 mL multiple dose cartridges)
Manufactured by [ ]
Lot No.: 97-0403KB
Expiration date: 10/17/2000

Subjects in Group 1 were assigned to the BID pramlintide treatments A-C in a randomized fashion, as follows:

Group 1: Treatment A: 60 µg , SC, BID, given just prior to breakfast and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (9 consecutive doses).

Treatment B: 120 µg, SC, BID, given just prior to breakfast and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (9 consecutive doses).

Treatment C: 180 µg, SC, BID, given just prior to breakfast and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (9 consecutive doses).

Subjects in Group 2 were assigned to the TID pramlintide treatments D-F in a randomized fashion, as follows:

Group 2: Treatment D: 60 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses).

Treatment E: 90 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a single dose prior to breakfast on the 5th day (13 consecutive doses).

Treatment F: 120 µg, SC, TID, given just prior to breakfast, lunch, and dinner for 4 days followed by a

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single dose prior to breakfast on the 5th day (13 consecutive doses).

PK MEASURES AND METHODS: Pramlintide doses were administered with [ ] Insulin Pen and the cartridge device [ ] Pharmacokinetic (pramlintide) and pharmacodynamic (glucose) assessments were made following administration of the first and last doses of each of the six subcutaneous pramlintide dosing regimens by measuring serial plasma concentrations from which the parameters C_{max}, T_{max}, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, AUCR, $t_{½}$, and Kel were calculated for pramlintide, and parameters C_{max}, T_{max}, C_{min}, T_{min}, and $AUC_{(0-300)}$ were calculated for baseline corrected glucose. At each dose level, subjective comparisons were performed between Day 1 and Day 5 parameters for the same dose level. Subjective dose proportionality assessment was performed for pramlintide C_{max} and $AUC_{(0-t)}$ for parameters of subjects within each group. For pramlintide, C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-inf)}$ parameter values were also normalized for individual Body Mass Index (BMI).

RESULTS: For the pharmacokinetic and pharmacodynamic analyses, data are presented for 12 subjects in the BID group who completed Treatments A, B, and C; and for 12 subjects who completed Treatments D and F and 11 subjects who completed Treatment E in the TID group.

Arithmetic means of the plasma pramlintide pharmacokinetic parameters for all 6 treatments are summarized in the following four tables.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

	Plasma Pramlintide					
	Treatment A N=12		Treatment B N=12		Treatment C N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	50.8	20.3	117.4	116.4	151.3	67.2
Cmax/BMI pmol/L	1.8	0.9	4.6	6.4	5.4	3.4
Tmax hr	0.331	0.107	0.341	0.105	0.368	0.221
AUC(0-t) pmol*hr/L	62.39	61.98	143.0	139.9	179.4	129.9
AUC(0-t)/BMI pmol*hr/L	2.163	2.069	5.509	6.990	6.310	5.138
AUC(0-inf) pmol*hr/L	119.1	86.42	201.6	160.0	201.0	140.4
AUC(0-inf)/BMI pmol*hr/L	4.045	2.570	7.750	8.205	7.048	5.488
T1/2 hr	1.24	0.774	1.24	0.832	0.926	0.496
Kel 1/hr	0.699	0.301	0.760	0.408	0.907	0.385
AUCR	0.748	0.144	0.838	0.0879	0.881	0.0438

Treatment A = 1 x 60µg Pramlintide BID
Treatment B = 1 x 120µg Pramlintide BID
Treatment C = 1 x 180µg Pramlintide BID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

	Plasma Pramlintide					
	Treatment A N=12		Treatment B N=12		Treatment C N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	55.9	24.6	97.8	34.0	137.3	36.4
Cmax/BMI pmol/L	2.0	1.4	3.5	1.8	4.8	1.6
Tmax hr	0.284	0.0723	0.324	0.124	0.339	0.224
AUC(0-t) pmol*hr/L	61.18	52.32	123.1	97.40	189.0	147.8
AUC(0-t)/BMI pmol*hr/L	2.158	2.007	4.321	3.674	6.452	5.175
AUC(0-inf) pmol*hr/L	133.7	67.68	163.4	111.0	232.1	166.2
AUC(0-inf)/BMI pmol*hr/L	4.557	2.421	5.701	4.140	7.924	5.814
T1/2 hr	1.52	1.09	1.02	0.502	0.981	0.406
Kel 1/hr	0.594	0.249	0.828	0.369	0.814	0.297
AUCR	0.731	0.106	0.839	0.0683	0.883	0.0197

Treatment A = 1 x 60µg Pramlintide BID
Treatment B = 1 x 120µg Pramlintide BID
Treatment C = 1 x 180µg Pramlintide BID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

	Plasma Pramlintide					
	Treatment D N=12		Treatment E N=11		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	36.4	18.5	55.7	25.7	74.0	24.6
Cmax/BMI pmol/L	1.3	0.8	1.9	1.2	2.6	1.1
Tmax hr	0.290	0.0723	0.253	0.00808	0.260	0.0303
AUC(0-t) pmol*hr/L	32.24	46.23	53.93	52.15	76.24	54.37
AUC(0-t)/BMI pmol*hr/L	1.164	1.697	1.924	2.032	2.693	2.095
AUC(0-inf) pmol*hr/L	201.5	111.6	124.9	85.32	119.2	79.45
AUC(0-inf)/BMI pmol*hr/L	7.532	3.259	4.592	3.145	4.231	2.974
T1/2 hr	2.72	1.49	1.38	1.20	1.10	0.778
Kel 1/hr	0.300	0.165	0.725	0.338	0.811	0.338
AUCR	0.620	0.0558	0.757	0.0832	0.788	0.0700

Treatment D = 1 x 60µg Pramlintide TID
Treatment E = 1 x 90µg Pramlintide TID
Treatment F = 1 x 120µg Pramlintide TID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

	Plasma Pramlintide					
	Treatment D N=12		Treatment E N=11		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	42.2	20.2	60.1	24.4	77.2	28.2
Cmax/BMI pmol/L	1.4	0.8	2.0	1.0	2.7	1.3
Tmax hr	0.255	0.00862	0.301	0.105	0.275	0.0712
AUC(0-t) pmol*hr/L	35.06	38.05	59.66	46.52	91.19	77.00
AUC(0-t)/BMI pmol*hr/L	1.235	1.471	2.070	1.784	3.252	2.942
AUC(0-inf) pmol*hr/L	107.7	67.12	129.2	63.29	152.4	102.7
AUC(0-inf)/BMI pmol*hr/L	3.813	2.682	4.550	2.519	5.532	3.852
T1/2 hr	1.42	1.40	1.43	0.991	1.15	0.543
Kel 1/hr	0.791	0.425	0.615	0.243	0.686	0.231
AUCR	0.755	0.100	0.760	0.0943	0.783	0.0527

Treatment D = 1 x 60µg Pramlintide TID
Treatment E = 1 x 90µg Pramlintide TID
Treatment F = 1 x 120µg Pramlintide TID

The arithmetic means of the baseline corrected plasma glucose pharmacodynamic parameters for all six treatments are summarized in the following four tables. Mean C_{max} values reflect the maximum magnitude of glucose increase following meal, while mean C_{min} values reflect the maximum glucose decrease. In the four cases where predose values were not available for baseline correction, no glucose response data are reported.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 1

	Baseline Corrected Plasma Glucose					
	Treatment A N=12		Treatment B N=12		Treatment C N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	109.99	43.03	82.06	36.77	103.73	39.54
Cmin(mg/dL)	-22.79	54.88	-34.82	42.23	-14.27	22.41
Tmax(hr)	2.53	0.923	2.33	1.21	2.91	0.490
Tmin(hr)	2.08	2.17	3.17	1.98	2.05	1.93
AUC(0-300)(mg*hr/dL)	219.6	193.4	135.7	165.3	220.2	110.1

Treatment A = 1 x 60µg Pramlintide BID
Treatment B = 1 x 120µg Pramlintide BID
Treatment C = 1 x 180µg Pramlintide BID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 5

	Baseline Corrected Plasma Glucose					
	Treatment A N=12		Treatment B N=10		Treatment C N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	91.83	60.15	94.42	59.21	54.59	51.96
Cmin(mg/dL)	-49.08	71.15	-31.46	52.01	-51.61	50.38
Tmax(hr)	1.84	1.04	2.60	1.17	2.14	1.36
Tmin(hr)	3.88	1.80	3.85	1.89	3.23	2.10
AUC(0-300)(mg*hr/dL)	122.0	335.2	170.4	262.8	25.33	242.8

Treatment A = 1 x 60µg Pramlintide BID
Treatment B = 1 x 120µg Pramlintide BID
Treatment C = 1 x 180µg Pramlintide BID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 1

	Baseline Corrected Plasma Glucose					
	Treatment D N=12		Treatment E N=11		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	120.01	45.50	110.15	33.78	93.03	43.22
Cmin(mg/dL)	8.93	20.39	-10.09	36.44	-4.69	9.77
Tmax(hr)	2.67	0.723	2.99	0.846	2.50	0.429
Tmin(hr)	0.542	0.144	1.39	1.75	0.910	1.27
AUC(0-300)(mg*hr/dL)	298.0	131.9	245.8	126.7	227.6	133.3

Treatment D = 1 x 60µg Pramlintide TID
Treatment E = 1 x 90µg Pramlintide TID
Treatment F = 1 x 120µg Pramlintide TID

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose, Day 5

	Baseline Corrected Plasma Glucose					
	Treatment D N=9		Treatment E N=9		Treatment F N=9	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(mg/dL)	31.44	45.84	103.11	52.06	89.37	33.80
Cmin(mg/dL)	-37.90	39.71	-19.98	43.33	-11.41	11.10
Tmax(hr)	2.11	0.558	3.22	0.793	2.78	1.06
Tmin(hr)	2.67	2.26	1.56	1.96	1.67	1.92
AUC(0-300)(mg*hr/dL)	187.5	187.2	286.4	195.3	207.8	112.0

Treatment D = 1 x 60µg Pramlintide TID
Treatment E = 1 x 90µg Pramlintide TID
Treatment F = 1 x 120µg Pramlintide TID

CONCLUSIONS:

- Pramlintide did not accumulate in the plasma of subjects with type 2 diabetes during the 5-day SC administration of either 60, 120, or 180 µg BID; or 60, 90, or 120 µg TID.
- Mean pramlintide concentration pharmacokinetic parameters C_{max} and $AUC_{(0-t)}$ increased relatively proportionally with increasing doses of pramlintide across the 60 to 180 µg dosing range.
- Following multiple dose administration of pramlintide (Day 5), the mean baseline corrected plasma glucose C_{max} for all treatment doses/regimens was similar to or lower than that observed on Day 1. The lack of a placebo group and the presence of confounding factors including insulin dosage timing relative to the meal, amount of meal consumed, and an incomplete record of insulin usage during the study periods make it difficult to draw pharmacodynamic conclusions.

1. PURPOSE

The original clinical study reports focused on the safety aspects of pramlintide treatment. In order to standardize the pharmacokinetic assessments across studies, a uniform analysis approach was applied in this addendum. While there are some specific differences between the analyses in this addendum and those in the clinical study report, the overall pharmacokinetic and pharmacodynamic findings and conclusions are generally similar.

The purpose of this addendum is 1) to provide additional analysis of pramlintide pharmacokinetics following each treatment after deletion of concentration values below the quantifiable limit (BQL values), 2) to provide additional pharmacokinetic and statistical analyses for pramlintide and insulin using an ANCOVA model with the predose baseline values as individual covariates, 3) to provide additional pharmacodynamic and statistical analyses of glucose data following subtraction of baseline (predose) glucose concentrations, and 4) to provide mean and individual plots of pramlintide, insulin, and glucose concentrations versus time data.

2. SYNOPSIS

The treatments were as follows:

Treatment A: 1 × 0.1 mL Placebo Plus Isophane Plus Soluble Insulin in One Syringe.

Treatment B: 1 × 30 µg/0.1 mL Pramlintide Plus Isophane in One Syringe and Soluble Insulin in a Separate Syringe.

Treatment C: 1 × 30 µg/0.1 mL Pramlintide Plus Soluble Insulin in One Syringe and Isophane in a Separate Syringe.

Treatment D: 1 × 30 µg/0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections.

Treatment E: 1 × 30 µg/0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe.

Comparisons of interest are summarized, by analyte, in the following table:

Pramlintide	Insulin	Glucose
B/D	A/D	E/A
C/D	B/D	E/D
E/D	C/D	B/D
	E/D	C/D
	A/E	

PK MEASURES AND METHODS: Pharmacokinetic and pharmacodynamic effects of co-administering pramlintide with insulin mixed together in the same syringe in various combinations, as specified, and as separate subcutaneous injections were assessed by measuring the concentrations of pramlintide, insulin, and glucose following each of the five treatments. Equivalent bioavailability of compared treatment pairs was shown for pramlintide and insulin if the 90% confidence intervals (CI) of the ratio of product means for Cmax, AUC(0-t), and AUC(0-inf) for pramlintide, and for Cmax, AUC(0-300), and AUC(0-600) for insulin were within the range of 80 to 125%. Pharmacodynamic effects on glucose were also assessed based by the comparison of Cmax, Tmax, Cmin, Tmin, and AUC(0-300) calculated from baseline-corrected glucose concentrations. (For definitions of abbreviations and symbols, see Section 3)

RESULTS: Data from all 29 subjects were included in the pharmacokinetic (insulin), pharmacodynamic (glucose) comparisons, and statistical analyses performed. Data from 28 subjects were included in the pramlintide comparisons and statistical analyses since data from Subject 1010 was not reported due to high background in the assay.

The arithmetic means of plasma pramlintide pharmacokinetic parameters and their statistical comparisons for Treatment B, Treatment C, and Treatment E versus Treatment D are summarized in the following 3 tables.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments B and D

	Plasma Pramlintide					
	Treatment B		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	33.21	20.20	42.43	20.32	64.0- 90.3	77.2
Cmax/BMI(pmol/L)	1.47	1.02	1.86	0.99	64.7- 91.5	78.1
Tmax(min)	32.3	15.4	18.5	9.07	. - .	.
AUC(0-t)(pmol*hr/L)	1513	1200	1626	1160	68.9-113.3	91.1
AUC(0-t)/BMI(pmol*min/L)	67.06	55.66	70.86	54.07	70.1-115.4	92.8
AUC(0-inf)(pmol*min/L)	4378	915.3	4274	1153	93.7-155.8	124.8
AUC(0-inf)/BMI(pmol*min/L)	192.2	45.13	187.7	48.65	95.7-154.5	125.1
T1/2(min)	45.4	13.8	47.1	28.6	31.3-147.2	89.2
Kel(1/min)	0.0164	0.00472	0.0173	0.00494	68.9-131.8	100.4
AUCR	0.674	0.0967	0.658	0.175	. - .	.
ln(Cmax)	3.523	0.4495	3.756	0.3702	68.8- 88.1	77.9
ln[AUC(0-t)]	7.090	1.011	7.279	0.6330	58.4-107.9	79.4
ln[AUC(0-inf)]	8.364	0.2362	8.329	0.2665	95.8-157.7	122.9

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus Isophane in One Syringe and Soluble Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 19.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments C and D

	Plasma Pramlintide					
	Treatment C		Treatment D			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	37.30	20.38	42.43	20.32	73.1- 99.4	86.2
Cmax/BMI(pmol/L)	1.61	0.96	1.86	0.99	71.8- 98.6	85.2
Tmax(min)	22.8	9.83	18.5	9.07	. - .	.
AUC(0-t)(pmol*hr/L)	1669	1383	1626	1160	76.7-121.1	98.9
AUC(0-t)/BMI(pmol*min/L)	71.55	61.99	70.86	54.07	74.6-119.9	97.2
AUC(0-inf)(pmol*min/L)	4495	1706	4274	1153	78.2-140.3	109.3
AUC(0-inf)/BMI(pmol*min/L)	187.8	85.48	187.7	48.65	81.3-140.0	110.6
T1/2(min)	51.7	14.6	47.1	28.6	40.6-156.6	98.6
Kel(1/min)	0.0143	0.00346	0.0173	0.00494	56.7-119.7	88.2
AUCR	0.625	0.143	0.658	0.175	. - .	.
ln(Cmax)	3.672	0.3596	3.756	0.3702	77.8- 99.6	88.0
ln[AUC(0-t)]	7.266	0.8262	7.279	0.6330	64.9-119.9	88.2
ln[AUC(0-inf)]	8.346	0.3855	8.329	0.2665	81.8-134.6	104.9

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Soluble Insulin in One Syringe and Isophane in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 20.

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide for Treatments E and D

Pharmacokinetic Parameters	Plasma Pramlintide: Treatment E Arithmetic Mean	SD	Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(pmol/L)	30.21	22.69	42.43	20.32	56.0- 82.4	69.2
Cmax/BMI(pmol/L)	1.34	1.02	1.86	0.99	56.5- 83.3	69.9
Tmax(min)	32.1	10.9	18.5	9.07	. - .	.
AUC(0-t)(pmol*hr/L)	1631	1451	1626	1160	73.9-118.4	96.2
AUC(0-t)/BMI(pmol*min/L)	71.82	63.97	70.86	54.07	74.5-119.8	97.2
AUC(0-inf)(pmol*min/L)	4805	1311	4274	1153	92.3-154.5	123.4
AUC(0-inf)/BMI(pmol*min/L)	210.8	51.03	187.7	48.65	93.6-152.4	123.0
T1/2(min)	52.2	15.6	47.1	28.6	72.5-188.5	130.5
Kel(1/min)	0.0142	0.00353	0.0173	0.00494	42.7-105.7	74.2
AUCR	0.636	0.0840	0.658	0.175	. - .	.
ln(Cmax)	3.631	0.4124	3.756	0.3702	69.4- 88.9	78.5
ln[AUC(0-t)]	7.413	0.9623	7.279	0.6330	66.7-123.3	90.7
ln[AUC(0-inf)]	8.441	0.2944	8.329	0.2665	94.2-155.1	120.9

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 21.

The arithmetic means of serum insulin pharmacokinetic parameters and their statistical comparisons for Treatment A, Treatment B, Treatment C, and Treatment E versus Treatment D are summarized in the following 4 tables.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments A and D

Pharmacokinetic Parameters	Serum Insulin: Treatment A Arithmetic Mean	SD	Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	118.44	205.00	84.59	45.27	78.4-187.0	132.7
Cmin(min)	26.74	23.75	28.54	21.86	73.3-105.3	89.3
Tmax(min)	195	91.2	139	98.6	. - .	.
Tmin(min)	279	286	325	254	. - .	.
AUC(0-300)(uU*min/mL)	21410	17098	18140	11523	97.6-131.5	114.5
AUC(0-600)(uU*min/mL)	36363	25726	30334	18892	103.9-130.0	116.9
ln(Cmax)	4.406	0.6359	4.324	0.4795	87.8-132.1	107.7
ln(Cmin)	3.066	0.6155	3.143	0.6421	71.0-107.8	87.5
ln[AUC(0-300)]	9.8155	0.49506	9.6820	0.46619	96.3-131.8	112.6
ln[AUC(0-600)]	10.364	0.47468	10.198	0.46726	101.6-132.9	116.2

Treatment A = 1 x 0.1 mL Placebo Plus Isophane Plus Soluble Insulin in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 48.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments B and D

Pharmacokinetic Parameters	Serum Insulin Treatment B Arithmetic Mean	SD	Serum Insulin Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	107.77	94.50	84.59	45.27	72.4-172.3	122.4
Cmin(min)	28.63	20.42	28.54	21.86	81.2-110.7	96.0
Tmax(min)	156	71.0	139	98.6	. - .	.
Tmin(min)	361	278	325	254	. .	.
AUC(0-300)(uU*min/mL)	24672	21978	18140	11523	113.0-144.1	128.6
AUC(0-600)(uU*min/mL)	38210	28801	30334	18892	109.3-133.3	121.3
ln(Cmax)	4.496	0.5353	4.324	0.4795	95.6-139.1	115.3
ln(Cmin)	3.155	0.6558	3.143	0.6421	78.3-114.9	94.8
ln[AUC(0-300)]	9.9266	0.53584	9.6820	0.46619	106.6-142.1	123.1
ln[AUC(0-600)]	10.406	0.48158	10.198	0.46726	105.8-135.4	119.7

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus Isophane in One Syringe and Soluble Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 49.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments C and D

Pharmacokinetic Parameters	Serum Insulin Treatment C Arithmetic Mean	SD	Serum Insulin Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	82.61	44.78	84.59	45.27	33.5-157.2	95.3
Cmin(min)	31.65	21.17	28.54	21.86	88.5-125.0	106.7
Tmax(min)	114	65.0	139	98.6	. - .	.
Tmin(min)	415	229	325	254	. - .	.
AUC(0-300)(uU*min/mL)	19073	11515	18140	11523	82.7-121.3	102.0
AUC(0-600)(uU*min/mL)	31375	20378	30334	18892	86.1-115.8	101.0
ln(Cmax)	4.322	0.4015	4.324	0.4795	77.7-123.7	98.0
ln(Cmin)	3.313	0.5119	3.143	0.6421	88.3-142.2	112.1
ln[AUC(0-300)]	9.7546	0.41259	9.6820	0.46619	87.7-125.4	104.9
ln[AUC(0-600)]	10.243	0.42900	10.198	0.46726	88.0-119.4	102.5

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Soluble Insulin in One Syringe and Isophane in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 50.

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments E and D

Pharmacokinetic Parameters	Serum Insulin Treatment E Arithmetic Mean	SD	Serum Insulin Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	84.60	45.01	84.59	45.27	44.5-155.0	99.7
Cmin(min)	24.64	16.59	28.54	21.86	68.8-101.4	85.1
Tmax(min)	227	84.1	139	98.6	. - .	.
Tmin(min)	221	282	325	254	. - .	.
AUC(0-300)(uU*min/mL)	18874	10885	18140	11523	86.7-121.2	104.0
AUC(0-600)(uU*min/mL)	33255	19638	30334	18892	96.7-123.2	110.0
ln(Cmax)	4.337	0.4341	4.324	0.4795	83.4-126.3	102.6
ln(Cmin)	3.066	0.4957	3.143	0.6421	73.9-113.1	91.4
ln[AUC(0-300)]	9.7380	0.43610	9.6820	0.46619	90.9-125.0	106.6
ln[AUC(0-600)]	10.305	0.43183	10.198	0.46726	97.9-128.7	112.3

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 51.

The arithmetic means of serum insulin pharmacokinetic parameters and their statistical comparisons for Treatment A versus Treatment E is summarized in the following table

Summary of the Pharmacokinetic Parameters of Serum Insulin for Treatments A and E

Pharmacokinetic Parameters	Serum Insulin Treatment A Arithmetic Mean	SD	Serum Insulin Treatment E Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(uU/mL)	118.44	205.00	84.60	45.01	77.9-188.3	133.1
Cmin(min)	26.74	23.75	24.64	16.59	85.9-124.1	105.0
Tmax(min)	195	91.2	227	84.1	. - .	.
Tmin(min)	279	286	221	282	. - .	.
AUC(0-300)(uU*min/mL)	21410	17098	18874	10885	93.7-126.7	110.2
AUC(0-600)(uU*min/mL)	36363	25726	33255	19638	94.3-118.4	106.4
ln(Cmax)	4.406	0.6359	4.337	0.4341	85.4-129.1	105.0
ln(Cmin)	3.066	0.6155	3.066	0.4957	77.4-118.3	95.7
ln[AUC(0-300)]	9.8155	0.49506	9.7380	0.43610	90.2-123.9	105.7
ln[AUC(0-600)]	10.364	0.47468	10.305	0.43183	90.4-118.6	103.5

Treatment A = 1 x 0.1 mL Placebo Plus Isophane Plus Soluble Insulin in One Syringe
Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe
* = Based on LS Means from Table 52.

The arithmetic means of baseline corrected plasma glucose pharmacodynamic parameters and their statistical comparisons for Treatments E versus A and Treatments E, B and C versus D are summarized in the following 4 tables.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments E and A

Pharmacokinetic Parameters	Plasma Glucose Treatment E Arithmetic Mean	SD	Plasma Glucose Treatment A Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	133	82	181	61	52.5- 93.2	72.8
Cmin(min)	-74	82	-91	74	109.7- 54.6	82.1
Tmax(min)	304	200	266	192	. - .	.
Tmin(min)	226	194	290	188	. - .	.
AUC(0-300)(mg*min/mL)	7035.9	27803	12726	22219	-24.5-121.3	48.4
AUC(0-600)(mg*min/mL)	22176	49960	26396	38067	8.2-152.3	80.3

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe
Treatment A = 1 x 0.1 mL Placebo Plus Isophane Plus Soluble Insulin in One Syringe
* = Based on LS Means from Table 79.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments E and D

Pharmacokinetic Parameters	Plasma Glucose Treatment E Arithmetic Mean	SD	Plasma Glucose Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	133	82	120	91	76.8-136.4	106.6
Cmin(min)	-74	82	-80	58	134.2- 66.8	100.5
Tmax(min)	304	200	282	178	. - .	.
Tmin(min)	226	194	257	210	. - .	.
AUC(0-300)(mg*min/mL)	7035.9	27803	2715.2	21250	-95.5-471.9	188.2
AUC(0-600)(mg*min/mL)	22176	49960	18961	45767	10.0-185.0	97.5

Treatment E = 1 x 30 ug in 0.1 mL Pramlintide, Isophane Plus Soluble Insulin All Mixed in One Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 80.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments B and D

Pharmacokinetic Parameters	Plasma Glucose Treatment B Arithmetic Mean	SD	Plasma Glucose Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	117	77	120	91	64.8-124.4	94.6
Cmin(min)	-90	66	-80	58	153.7- 86.3	120.0
Tmax(min)	368	198	282	178	. - .	.
Tmin(min)	244	162	257	210	. - .	.
AUC(0-300)(mg*min/mL)	-3489.2	23468	2715.2	21250	-447-120.9	-163.0
AUC(0-600)(mg*min/mL)	10611	44414	18961	45767	-45.7-129.5	41.9

Treatment B = 1 x 30 ug in 0.1 mL Pramlintide Plus Isophane in One Syringe and Soluble Insulin in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 81.

Summary of the Pharmacodynamic Parameters of Baseline Corrected Plasma Glucose for Treatments C and D

Pharmacokinetic Parameters	Plasma Glucose Treatment C Arithmetic Mean	SD	Plasma Glucose Treatment D Arithmetic Mean	SD	90% CI*	% Mean Ratio*
Cmax(mg/mL)	142	105	120	91	83.9-143.4	113.6
Cmin(min)	-87	80	-80	58	150.0- 82.7	116.3
Tmax(min)	356	178	282	178	. - .	.
Tmin(min)	213	154	257	210	. - .	.
AUC(0-300)(mg*min/mL)	-812.11	26466	2715.2	21250	-365-202.8	-80.9
AUC(0-600)(mg*min/mL)	20745	57503	18961	45767	2.7-177.7	90.2

Treatment C = 1 x 30 ug in 0.1 mL Pramlintide Plus Soluble Insulin in One Syringe and Isophane in a Separate Syringe
Treatment D = 1 x 30 ug in 0.1 mL Pramlintide, Soluble Insulin and Isophane Each as Separate Injections
* = Based on LS Means from Table 82.

CONCLUSIONS:

- Mixing pramlintide with Isophane and/or soluble insulin in a single syringe substantially reduced the rate of pramlintide absorption compared to administering each in separate syringe (Treatment D). The extent of absorption also decreased, but the effects were generally less pronounced. The decreases in mean ratios for Cmax were 22.1%, 12%, and 21.5% for the comparisons of Treatment B, Treatment C, and Treatment E versus Treatment D, respectively. Mean ratios for the respective comparisons of AUC(0-t) were 20.6%, 11.8%, and 9.3%. Neither Treatment B, Treatment C, or Treatment E could be considered bioequivalent with Treatment D.

- Comparison of placebo versus pramlintide mixed in the same syringe with the two insulin types (Treatment A versus Treatment E) showed that addition of pramlintide had little effect on insulin rate and extent of absorption. The mean ratios of Cmax, AUC(0-300), and AUC(0-600) were 105.0%, 105.7%, and 103.5%. However, compared to administering pramlintide and each insulin type in separate syringe (Treatment D), placebo mixed in the same syringe with the two insulin types resulted in increased rate and extent of insulin availability

- Mixing pramlintide with Isophane and/or soluble insulin in a single syringe had variable effects on insulin pharmacokinetics. Co-administration of soluble insulin in the same syringe with pramlintide (Treatment C) had no effects on the rate or extent of insulin absorption compared to reference Treatment D. Similarly, co-administration of both Isophane and soluble insulin in the same syringe with pramlintide (Treatment E) had little effect on insulin rate and extent of absorption, at least within the first 300 min. Co-administration of Isophane in the same syringe with pramlintide (Treatment B) resulted in an increase in both the rate and extent of insulin absorption. Due to the large variability in individual parameters, the 90% CI for all treatment pairs were wide, extendeding outside the acceptable 80% - 125% range. Consequently, none of these four treatments could be considered bioequivalent with Treatment D.

- Comparison of mean baseline corrected concentration time profiles for glucose indicates that addition of pramlintide to insulin, even if the two insulin types and pramlintide are mixed in the same syringe, essentially abolishes the effect of food by maintaining glucose concentrations at or below baseline levels through at least the first 300 min after the meal. Administering pramlintide in a separate syringe from the two insulin types shows no substantial differences in mean Cmax of baseline corrected glucose concentrations; however, the baseline corrected AUC(0-300) was almost twice as high for Treatment E compared with Treatment D (Table 80). When non-baseline corrected glucose AUC (0-300) was anlyazed, no differences were observed between the two treatments (mean ratio = 97.7%).

- Comparisons of Treatment B and Treatment C with Treatment D showed that, based on baseline corrected glucose data, mixing pramlintide with one of the insulins also overall reduced the mean glucose Cmax and AUC(0-300) compared to when pramlintide and both insulins were given as three separate injections. The effect of mixing Isophane with pramlintide (Treatment B) was more pronounced compared to when soluble insulin was mixed with pramlintide (Treatment C).

- Because of the variable and inconsistent effect of mixing on pramlintide and insulin concentrations, it is not recommended that Isophane and/or soluble insulin be mixed with pramlintide the same syringe prior to administration; however, there is no indication that mixing results in any undue risk to patients with regard to glucose control.

3. PHARMACODYNAMIC AND STATISTICAL MEASURES AND METHODS

Although the lower limit of quantitation (LLOQ) for pramlintide for the IEMA assay is — pmol/L, due to the standards used, the actual limit of quantitation for this study, referred to as BQL, was — pmol/L. Any value below that limit has been reset to zero in the concentration tables and for the purpose of pharmacokinetic analysis.

The following pharmacokinetic parameters were calculated from the pramlintide concentrations:

- Cmax — Maximum observed concentration within the sampling interval obtained without interpolation.
- Tmax — Time of the maximum observed concentration.
- AUC(0-t) — Area under the drug concentration-time curve from time zero to time t, AUC(0-t), where t is the time of the last measurable concentration (Ct).
- AUC(0-inf) — Area under the drug concentration-time curve from time zero to infinity, AUC(0-inf) = AUC(0-t) + Ct/Kel, where Kel is the terminal elimination rate constant.
- AUCR — The ratio of AUC(0-t) to AUC(0-inf).
- T1/2 — Effective elimination half-life, calculated as ln(2)/Kel.
- Kel — Apparent first-order elimination or terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-squares regression analysis using the last three (or more) non-zero plasma concentrations.

In addition, individual parameters Cmax, AUC(0-t), and AUC(0-inf) were corrected for Body Mass Index (BMI).

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-118

Title of Study: The Effect of Single Doses of Pramlintide on the Gastric Emptying of Two Meals

Investigators and Study Centers: R.B. Tattersall, Department of Diabetes, University Hospital, Queen's Medical Centre, Nottingham, United Kingdom

Publication (Reference): Kong M-F, Stubbs TA, King P, Macdonald IA, Lambourne JE, Blackshaw PE, Perkins AC, Tattersall, RB. The effect of single doses of pramlintide on gastric emptying of two meals in men with IDDM. Diabetologia 1998;41:577-583.

Studied Period: April 1996 - August 1996 | Phase of Development: 2

Objectives: The primary objective was to determine the dose-response relationship of single doses of pramlintide (30, 60, and 90 µg) on the rate of gastric emptying of the liquid and solid components of a standard meal and to establish if a dose of pramlintide administered before breakfast has a "carry-over" effect on the emptying of the lunchtime meal.

Methodology: The study was a single-blind, randomized, four-way crossover comparison of three dose levels of pramlintide and placebo administered subcutaneously to subjects with type 1 diabetes. Each period began the evening before dosing, and timed study evaluations continued up to 480 minutes post-dose. A breakfast meal was administered 15 minutes after dosing and a lunch meal was administered 4 hours later.

Number of Subjects: Fourteen subjects with type 1 diabetes were randomly assigned to one of four treatment sequences. The subjects were all white males, ranging in age from 20 to 42 years old. The overall range of diabetes duration was 0.8 to 27.5 years. Baseline HbA_{1c} ranged from 6% to 15%. Baseline C-peptide concentration ranged from <30 pmol/L to 52 pmol/L for 13 of the 14 subjects, and was 456 pmol/L in the one subject with recently (0.8 years) diagnosed type 1 diabetes. All 14 subjects were dosed with study medication and completed the study. Of the 14 subjects, 3 received an insulin infusion during test period and were considered unevaluable for pharmacokinetic and pharmacodynamic analyses. All 14 subjects were evaluated for safety and constitute the intent-to-treat population.

Diagnosis and Main Criteria for Inclusion: Males between 18 and 50 years of age with a history of type 1 diabetes mellitus (as documented by a history of diabetic ketoacidosis or a C-peptide concentration ≤1.0 ng/mL [331 pmol/L]) for at least two years but not exceeding 20 years. In addition, the subjects must have met all inclusion and exclusion criteria specified in the protocol, and must have followed protocol-specified restrictions throughout the study.

Test Product, Dose and Mode of Administration, Batch No.: 95-0902GB (Final Drug Product [FDP]). Pramlintide 0.3 mg/mL (AC-0137-F21) for subcutaneous injection formulated as sterile liquid in glass vials containing mannitol, metacresol, and adjusted to pH 4.0 with acetic acid and sodium acetate trihydrate. The test product was diluted as necessary with reference product, so that for each dose level of pramlintide an injection volume of 0.3 mL provided the requisite amount of active drug.

Duration of Treatment: A single dose of study medication (pramlintide 30 µg, 60 µg, or 90 µg, or placebo) was self-administered during each of the four crossover periods according to the single-blind randomization schedule. A washout of 7 to 14 days was scheduled between treatment periods. Subjects returned to the clinic for a medical evaluation 10 to 14 days after the final treatment.

Reference Therapy, Dose and Mode of Administration, Batch No: 95-0504GE. A single subcutaneous injection of placebo (0.3 mL) was self-administered by subjects during one of the four crossover periods according to the single-blind randomization schedule. Placebo was provided by the Sponsor as a Reference Product Formulation identical to test product, except containing no pramlintide.

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Criteria for Evaluation:

Pharmacodynamic Evaluation: Gastric emptying of solid content following the first meal was assessed by calculating the percent of radioactive label remaining in the stomach at timed intervals after a ^{99m}Technetium-labeled standard meal (breakfast). Gastric emptying following a second meal was based on the percent label remaining in the stomach following an ^{111}Indium-labeled standard meal (lunch). Gamma camera imaging provided counts per minute that were adjusted for background and decay and were used to derive the percent label remaining. Zero time for all evaluations was the start of the breakfast meal. The subject's regular insulin dose was self-administered at -30 minutes, and the single dose of study drug at -15 minutes relative to this meal. The standard breakfast and lunch meals included liquid containing 3-ortho-methyl glucose (3-OMG), a non-metabolized sugar which served as a surrogate marker for the emptying of the liquid contents of the meals.

Pharmacokinetics: Plasma pramlintide concentrations were measured at selected times up to 480 minutes after the start of breakfast (495 minutes after dosing with pramlintide). PK parameters ($AUC_{0\text{-}240\ min}$, $AUC_{240\text{-}480\ min}$, C_{max}, and T_{max}) were calculated using the time of the breakfast meal as time zero (t=0).

Safety: Safety evaluations included adverse event monitoring, clinical laboratory assessments, physical examinations, electrocardiograms, and vital signs (pulse rate and systolic and diastolic blood pressure (BP)).

Statistical Methods: AUCs, C_{max}, and T_{max} were calculated from the concentration over time profiles for plasma pramlintide, blood glucose, plasma 3-OMG, and serum insulin. The pramlintide AUCs, C_{max}, and T_{max}, and the total stomach emptying (%); the half-emptying times (t_{50}) in minutes; and the lag times (min), for the first meal and second meal data, were analyzed using an ANOVA model for a 4 x 4 crossover trial with parameters for subjects, periods, treatments, and first-order carryover effects. Treatment differences, t values, p-values and 95% confidence intervals for treatment differences between each dose group and placebo, and between dose groups constructed from the adjusted means (LS means) and error mean square of ANOVA from SAS® PROC GLM, were also reported. Individual and mean profiles by treatment group were plotted for concentration over time data of the evaluable subjects for post-prandial glucose profiles, plasma pramlintide profiles, serum insulin profiles, 3-OMG plasma profiles, and rate of gastric emptying of solid component of meal. Descriptive summary statistics (n, mean, standard deviation, median, minimum, and maximum) were also tabulated. Individual subjects half-emptying times (t_{50}, in minutes) of solid component of the first meal and of the second meal were plotted against dose for each meal separately.

SUMMARY – CONCLUSIONS:

PHARMACODYNAMIC RESULTS:

Gastric Emptying of Liquid Food (3-OMG): The appearance of 3-OMG in plasma after breakfast was delayed after administration of all three doses of pramlintide compared with placebo. However, the morning dose of pramlintide had no effect on the subsequent appearance in plasma of the 3-OMG ingested with lunch. After breakfast (the first meal), compared with placebo, each of the three doses of pramlintide brought about a statistically significant ($p \leq 0.0006$) prolongation in the time to peak plasma concentrations of 3-OMG (T_{max}), and corresponding statistically significant ($p \leq 0.0057$) decreases in the $AUC_{(0\text{-}240\ min)}$ and C_{max} of 3-OMG. After lunch (the second meal), none of the pramlintide vs placebo comparisons were statistically significant.

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PHARMACODYNAMIC RESULTS (continued):

Gastric Emptying of Solid Food: The percent ^{99m}Tc label remaining in the stomach after breakfast was greater after all morning doses of pramlintide compared to placebo during the 240-minute assessment period. Both the half-emptying time and the lag time of the solid component of breakfast (based on ^{99m}Tc) were prolonged in an apparent dose-related manner after a morning dose of pramlintide. Compared with placebo, each of the three doses of pramlintide resulted in a statistically significant delay in both the half-emptying time and the lag time of the solid component of breakfast ($p \leq 0.0002$ for half-emptying time and $p \leq 0.0173$ for lag time). In contrast to pramlintide-induced delays in gastric emptying seen after the breakfast meal, no such delays were seen after the lunch meal. No significant differences between placebo and any of the pramlintide doses were observed for half-emptying time or lag time of the solid component of lunch (based on ^{111}In). Gastric emptying results showing the gastric emptying of solid foods are summarized below.

Gastric Half-Emptying Time and Lag Time by Treatment Group, Population: Evaluable (N=11)

	Placebo	Pramlintide 30 µg	Pramlintide 60 µg	Pramlintide 90 µg
BRAKFAST (first meal)				
Half-Emptying Time (min)				
arithmetic mean	128.6	187.2	200.1	214.5
adjusted mean (SE)	129.0 (9.8)	187.8 (9.8)	198.3 (9.8)	215.3 (9.8)
difference vs placebo	NA	58.9	69.4	86.3
p-value[1] vs placebo	NA	0.0002	0.0001	0.0001
Lag Time (min)				
arithmetic mean	32.5	54.4	56.4	70.3
adjusted mean (SE)	32.1 (6.2)	54.6 (6.2)	55.6 (6.2)	71.2 (6.2)
difference vs placebo	NA	22.4	23.5	39.1
p-value[1] vs placebo	NA	0.0173	0.0130	0.0001
LUNCH (second meal)				
Half-Emptying Time (min)				
arithmetic mean	136.9	138.5	140.6	145.4
adjusted mean (SE)	136.9 (5.4)	139.4 (5.4)	139.4 (5.4)	145.7 (5.4)
difference vs placebo	NA	2.4	2.4	8.8
p-value[1] vs placebo	NA	0.7497	0.7497	0.2590
Lag Time (min)				
arithmetic mean	34.2	46.5	40.0	48.2
adjusted mean (SE)	34.0 (6.7)	46.8 (6.7)	39.4 (6.7)	48.7 (6.7)
difference vs placebo	NA	12.7	5.4	14.7
p-value[1] vs placebo	NA	0.1938	0.5772	0.1353

[1] p-values from t-tests for multiple comparisons following ANOVA
NA=not applicable

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PHARMACOKINETIC RESULTS: The mean (SD) plasma pramlintide concentrations at 0 time (15 minutes after subcutaneous injection of pramlintide) were 36.4 (22.7), 59.3 (40.1), and 96.6 (42.5) pmol/L after doses of 30, 60, and 90 µg pramlintide, respectively. At 60 minutes (75 minutes after subcutaneous injection of pramlintide), mean (SD) plasma pramlintide concentrations were 21.7 (14.6), 28.1 (14.4), and 47.5 (34.6) pmol/L for pramlintide 30, 60, and 90 µg treatments, respectively. By 120 minutes (135 minutes after subcutaneous injection of pramlintide), mean (SD) plasma pramlintide concentrations had declined, but were still measurable: 11.8 (9.3), 15.0 (10.4), and 24.4 (22.9) pmol/L for pramlintide 30, 60, and 90 µg treatments, respectively; and at 240 minutes (255 minutes after subcutaneous injection of pramlintide) the corresponding mean (SD) pramlintide concentrations were 1.5 (2.6), 4.2 (4.5), and 6.6 (9.5) pmol/L. $AUC_{(0\text{-}240\ min)}$ and C_{max} increased with increasing doses of pramlintide, whereas T_{max} was essentially unaffected by dose. Pairwise comparisons between treatments for $AUC_{(0\text{-}240\ min)}$ showed a statistically significant difference between the 90 µg dose and each of the other two doses: for 90 µg vs 60 µg, p=0.0148 and for 90 µg vs 30 µg, p=0.0010. The difference in $AUC_{(0\text{-}240\ min)}$ between the 60 µg and 30 µg treatments was not statistically significant (p=0.1802). For C_{max}, all the pairwise comparisons between the three pramlintide doses showed statistically significant differences ($p \leq 0.0114$). None of the pairwise comparisons were statistically significant for T_{max} ($p \geq 0.7967$).

SAFETY RESULTS: Pramlintide was well-tolerated in this study. The overall incidence of adverse events was similar after administration of placebo, 60 µg or 90 µg pramlintide (28.6% each) and lower after administration of 30 µg pramlintide (14.3%). Nausea was the most common event, reported by 6 subjects (42.9%). The next most common was hypoglycemia, reported by 5 subjects (35.7%). Other reported events included dizziness, diarrhea, hyperglycemia, and inflicted injury. All events were mild or moderate in intensity, none was severe. There were no deaths or other serious adverse events, and no subject discontinued treatment due to an adverse event. There were no clinical laboratory findings, vital signs results, physical examination findings, or ECG findings of clinical concern.

CONCLUSION: This study demonstrates that a subcutaneously administered single dose of 30, 60, or 90 µg of pramlintide in subjects with type 1 diabetes mellitus results in a significant delay of gastric emptying for both liquid and solid foods. The delay in gastric emptying is limited to the meal following drug administration, with no carry over to a meal ingested 4.25 hours later. Pramlintide's effects on gastric emptying occur at plasma pramlintide concentrations similar to the physiological concentrations of endogenous amylin found post-prandially in healthy subjects. Pramlintide is well tolerated at the dosages used in this study.

Date of the Report: 20 June 2000

SYNOPSIS

NAME OF SPONSOR/COMPANY: Amylin Pharmaceuticals, Inc. NAME OF FINISHED PRODUCT: pramlintide injection NAME OF ACTIVE INGREDIENT(S): pramlintide acetate	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Protocol No.: 137-137

Title of Study: A Study to Determine the Effect of Pramlintide on the Gastric Emptying of Subjects with Type 2 Diabetes Mellitus Currently Requiring Insulin

Investigators and Study Centers: ⊏ ⅃

Publication (Reference): None

Studied Period: February 1998 – June 1998 | **Phase of Development:** 2

Objectives: The primary objective of this study was to compare the effect of pramlintide with placebo on gastric emptying rates for solid components of a standard breakfast meal, following a single subcutaneous dose. The comparison between pramlintide and placebo was to be made in a cross-over design.

The secondary objectives were to determine the safety of pramlintide when administered to this group of subjects and to determine the effect of pramlintide compared to placebo on motilin, glucagon, C-peptide, triglycerides and pancreatic polypeptide concentrations.

Methodology: This was a single-center, double-blind, randomized, crossover study comparing a single subcutaneous 90 µg dose of pramlintide to placebo on the rate of gastric emptying of the solid component of a standard breakfast meal. Gastric emptying was assessed by radiolabeling the test meal with technetium 99m (^{99m}Tc) and monitoring by gamma camera the percent of label remaining in the stomach over time.

Number of Subjects: Ten subjects (five males, five females) with a mean age of approximately 66 years were enrolled in this study. All subjects completed the study.

Diagnosis and Main Criteria for Inclusion: Males and females between 35 and 75 years of age with a history of type 2 diabetes mellitus (no past history of diabetic ketoacidosis), requiring treatment with insulin for a period of 6 months or more at study screening. In addition, the subjects were to have fulfilled the requirements of all inclusion and exclusion criteria specified in the protocol, and were to have followed protocol-specified restrictions throughout the study.

Test Product, Dose and Mode of Administration, Batch No.: Pramlintide (AC-0137-F24), 0.1 mL of 0.9 mg/mL (90 µg) administered subcutaneously, Lot No. 96-1002JB.

Duration of Treatment: A single dose of study medication (pramlintide 90 µg or placebo) was administered during each of the two crossover periods. A washout period of 7 to 14 days was scheduled between treatment periods.

Reference Therapy, Dose and Mode of Administration, Batch No: Placebo identical to test product except containing no pramlintide, 0.1 mL administered subcutaneously, Lot No. 97-0101GE

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Criteria for Evaluation:

Pharmacodynamics: Gastric emptying of solid content following a meal was assessed by calculating the percent of radioactive label remaining in the stomach at timed intervals after a ^{99m}Tc-labeled standard meal (breakfast). Time zero for all evaluations was the start of the breakfast meal. The subject's regular insulin dose was self-administered at -30 minutes, and the single dose of study drug at -15 minutes relative to this meal. Concentrations of circulating blood glucose, plasma motilin, plasma glucagon, serum C-peptide, serum triglycerides, and plasma pancreatic polypeptide were measured at selected times up to 300 minutes after the start of breakfast to determine the effect of pramlintide versus placebo on the various analytes.

Pharmacokinetics: Plasma pramlintide and serum insulin concentrations were measured at selected times up to 300 minutes after the start of breakfast (315 minutes after dosing with pramlintide). Pharmacokinetic parameters ($AUC_{20\text{-}300\ min}$, C_{max}, and T_{max}) were calculated using the time of the breakfast meal as time zero (t=0).

Safety: Safety evaluations included adverse event monitoring, clinical laboratory assessments, physical examinations, ECGs, and vital signs (pulse rate and systolic and diastolic blood pressure).

Statistical Methods:

Gastric half-emptying time (T_{50}) was defined as the time in minutes at which 50% of the radioisotope (^{99m}Tc) had left the stomach. Gastric lag time quantifies the initial phase of gastric emptying and was defined as the time in minutes required for 10% of the ingested isotope to leave the stomach. AUC, C_{max}, and T_{max} were calculated from the concentration over time profiles for plasma pramlintide, blood glucose, serum insulin, plasma motilin, plasma glucagon, serum C-peptide, serum triglycerides and plasma pancreatic polypeptide.

The variables T_{50}, T_{10}, AUC, C_{max}, and T_{max} were analyzed using standard analysis of variance (ANOVA) models for the 2 x 2 crossover design. If preliminary exploratory analysis of the summary measures data showed that ANOVA assumptions were strongly violated, an alternative non-parametric approach was used.

Summary statistics including sample size (N), arithmetic means, adjusted means (in case of imbalance), and standard errors of means or adjusted means were given for each treatment group. Treatment differences, t-values, p-values and 95% confidence intervals for treatment differences between the pramlintide and placebo groups, were also reported.

Individual and mean profiles by treatment group were plotted for concentration-time data from the evaluable subject population (all randomized subjects were considered evaluable) for blood, plasma, and serum analytes and for percent of ^{99m}Tc remaining in the stomach. Descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) were also performed.

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SUMMARY – CONCLUSIONS:

PHARMACODYNAMIC RESULTS: Pramlintide 90 µg administered by subcutaneous injection prior to a meal significantly delays gastric emptying of the solid food contents in subjects with type 2 diabetes mellitus using insulin. Compared with placebo, pramlintide 90 µg resulted in a statistically significant delay in both half-emptying time (T_{50}) and the lag time (T_{10}) of the solid component of a meal (p=0.0011 for half-emptying time and p=0.0232 for lag time).

Gastric Emptying Parameters, Population: Evaluable (N=10)

	Placebo	Pramlintide 90 µg
Half-Emptying Time (min)		
arithmetic mean	80.50	127.00
adjusted mean (SE) [1]	79.79 (6.69)	126.67 (6.69)
p-value [2]	NA	0.0011
Lag Time (min)		
arithmetic mean	17.00	28.80
adjusted mean (SE) [1]	17.29 (3.33)	30.46 (3.33)
p-value [2]	NA	0.0232

[1] from fitting a standard analysis of variance model for 2 X 2 crossover design
[2] p-value from standard ANOVA for 2 X 2 crossover
NA = not applicable

Pramlintide 90 µg significantly decreases postprandial plasma pancreatic polypeptide concentrations, and delays its appearance.

No changes in motilin concentrations were observed, suggesting that this hormone is unlikely to mediate the effects of pramlintide on gastric emptying.

SAFETY RESULTS:

Adverse Events: Pramlintide was well-tolerated in this study. One subject reported two incidences of diarrhea, one during the placebo treatment period and one during the pramlintide treatment period. Both events were mild and considered by the investigator to probably not be related to the study medication.

Deaths: There were no deaths.

Serious Adverse Events: There were no serious adverse events.

Clinical Laboratory Values: There were no laboratory findings of clinical concern.

Vital signs, Physical Examinations and ECGs: There were no marked changes in the mean values of systolic or diastolic blood pressure or heart rate over time, and no apparent differences after the administration of pramlintide as compared to placebo. No clinically important physical examination changes from baseline were reported for any subject during the study. ECGs were only recorded at screening, at which time two subjects had overall ECG interpretations that were considered abnormal but not clinically important.

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CONCLUSION: Pramlintide, administered subcutaneously prior to a meal as a single 90 µg dose to subjects with type 2 diabetes mellitus using insulin results in a significant delay in gastric emptying of the solid component of a meal. Pramlintide 90 µg significantly decreases the amount of circulating pancreatic polypeptide present after the meal, and delays its appearance. The lack of changes in plasma motilin concentrations suggests that this hormone is unlikely to mediate the effects of pramlintide on gastric emptying. Pramlintide is well tolerated when given as a single 90 µg dose to subjects with type 2 diabetes mellitus using insulin.		
Date of the Report: 27 September 2000		

22 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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Steve Johnson
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Hae-Young Ahn
9/7/01 05:12:11 PM
BIOPHARMACEUTICS

New Drug Application Filing Memorandum

Office of Clinical Pharmacology and Biopharmaceutics

NDA:	21-332	**Priority Classification:**	
IND:	39,897	**Indication:**	Diabetes Mellitus
Brand Name:	Symlin™ Injection	**Submission Date:**	7-DEC-00
Generic Name:	Pramlintide Acetate	**Route of Administration:**	SC Injection
Sponsor:	Amylin Pharmaceuticals	**UFGD:**	7-OCT-01
Reviewer:	Steven B. Johnson, BSPharm, PharmD	**Review Division:**	870
Team Leader:	Hae-Young Ahn, PhD	**Medical Division:**	510

Items included in NDA (CTD)	*Yes*	*No*	*Request*
Table of Contents present and sufficient to locate reports, tables, data, etc.	X		
Tabular Listing of All Human Studies	X		
HPK Summary	X		
Study Synopsis	X		
Labeling	X		
Bioavailability and Bioequivalence Studies:			
ADME Study –			
BA Studies –			
Absolute BA	X		
Relative BA	X		
BE Studies –			
Average BE	X		
Population BE		X	
Individual BE		X	
Food-Drug Interaction Study		X	
In Vitro-In Vivo Comparison (IVIVc) Studies		X	
Reference Bioanalytical and Analytical Methods	X		
Dissolution Profiles		X	
Studies Using Human Biomaterials:			
Plasma Protein Binding Studies	X		
Metabolism Studies Using Hepatocytes, Microsomes, etc.	X		
Blood / Plasma Ratio	X		
Human Pharmacokinetics (PK) Studies:			
PK and Initial Safety and Tolerability in Healthy Volunteers –			
Single Dose	X		
Multiple Dose	X		
PK and Initial Safety and Tolerability in Patient Volunteers –			
Single Dose	X		
Multiple Dose		X	
Dose Proportionality –			
Single Dose	X		
Multiple Dose		X	
PK in Population Subsets to Evaluate Intrinsic Factor Effects –			
Ethnicity		X	
Gender		X	
Pediatrics		X	
Geriatrics		X	
Renal Impairment	X		
Hepatic Impairment		X	
PK in Population Subsets to Evaluate Extrinsic Factor Effects –			
In-Vivo Effects on Primary Drug		X	
In Vivo Effects of Primary Drug	X		
In-Vitro Drug Interaction		X	

Population PK Studies		X	
Summary of PK / PD Studies	X		
PK / PD Studies in Volunteers		X	
PK / PD Studies in Patients	X		
Individual Datasets for all PK and PK / PD Studies in Electronic Format	X		
Other:			
Genotype / Phenotype Studies		X	
Chronopharmacokinetics		X	
Literature – Number of Articles Sufficient	X		

Additional Notes:

- Symlin™ is a new therapeutic class called amylinomimetics or amylin receptor agonists.
- Symlin™ is scheduled for the July, 2001 Advisory Committee Meeting
- This review will be on a 10 month review cycle.

Which Phase IV Studies Requested?

- **NONE**

This Application is filable.

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16 JAN 2001

Steven B. Johnson, B.S.Pharm., Pharm.D.; FDA / CDER / OPS / OCPB / DPE-II

1/17/01

Hae-Young Ahn, Ph.D., Team Leader; FDA / CDER / OPS / OCPB / DPE-II

CC: NDA 21-332; HFD-850 (LeskoL, LeeP); HFD-870 (MalinowskiH, AhnH, JohnsonST); CDR

Symlin™ Injection
(pramlintide acetate)

Item 6: Human Pharmacokinetics and Bioavailability (HPBIO)

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