Featured Article

An amylin analog used as a challenge test for Alzheimer's disease

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Abstract

Introduction: Preclinical studies demonstrate the potential of amylin in the diagnosis of Alzheimer's disease (AD). We aimed to lay the foundation for repurposing the amylin analog and a diabetes drug, pramlintide, for AD in humans.

Methods: We administered a single subcutaneous injection of 60 μg of pramlintide to nondiabetic subjects under fasting conditions.

Results: None of the participants developed hypoglycemia after the injection of pramlintide. The pramlintide challenge induced a significant surge of amyloid-β peptide and a decrease in total tau in the plasma of AD subjects but not in control participants. The pramlintide injection provoked an increase in interleukin 1 receptor antagonist and a decrease in retinol-binding protein 4, which separates AD subjects from control subjects.

Discussion: Pramlintide use appeared to be safe in the absence of diabetes. The biomarker changes as a result of the pramlintide challenge, which distinguished AD from control subjects and mild cognitive impairment.

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Keywords: Alzheimer's disease (AD); Amylin challenge; Pramlintide; Safety; Diagnosis; Biomarkers

1. Introduction

Currently, lumbar punctures to detect biomarkers in cerebrospinal fluid (CSF) [1] and positron emission tomography (PET) imaging scans [2,3] are used to diagnose Alzheimer's disease (AD). Despite their clinical utility in diagnosing AD in living patients, the use of lumbar puncture to obtain CSF samples is unacceptable for many patients because of a fear of the procedure and PET scans are costly. As the number of patients with AD is rapidly increasing, we urgently need blood tests that are simple and specific and could be easily performed in a doctor's office to diagnose AD.

However, there are many challenges associated with developing blood diagnostic tests to detect AD. Several studies show differences in plasma amyloid-β peptide (Aβ) between AD and control subjects with statistical significance [4,5]; however, the sensitivity and specificity are too low for AD diagnosis [6–8]. The blood levels of inflammatory factors show significant differences between AD and control subjects [9]; however, they are also insufficient for AD diagnosis. The existence of the blood-brain

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barrier (BBB) [10,11] is the major challenge of developing blood tests for AD. If a drug can mobilize AD biomarkers from the brain into blood, the sensitivity and specificity of these AD biomarkers in the test can be enhanced to reveal the brain pathology. These kinds of drugs may also become therapeutic for AD.

Amylin, a gut-brain axis hormone with 37 amino acids, is produced and secreted by the pancreas. Because it crosses the BBB [12,13], amylin may mediate brain functions including regulating glucose metabolism [14] and modulating inflammation [15,16], as it does in the peripheral system. Using AD mouse models, two independent studies including our own demonstrate that treatment with amylin or its clinical analog, pramlintide, reduces the AD pathology in their brains and improves their learning and memory functions [17,18]. A peripheral injection of amylin or pramlintide causes a removal of Aβ from the brain into blood [17]. Taken together, it prompted us to develop (1) the therapeutic for AD and (2) an amylin challenge test to diagnose AD, similar to the glucose tolerance test to diagnose type 2 diabetes.

Pramlintide is a synthetic version of naturally occurring amylin with three amino acid changes and is an Food and Drug Administration-approved drug for diabetes that has a favorable safety profile [19]. Therefore, pramlintide could be repurposed for AD as a novel alternative therapeutic. Because most patients with AD do not have diabetes but often have poor appetite and caloric intake, it is necessary to develop an evidence-based support to repurpose this drug for AD before a large study is conducted. We report here the results of a pilot trial where we administered a single subcutaneous injection of pramlintide to human subjects. The study has two parts: the first stage was to examine the safety profile of pramlintide in the absence of diabetes and under fasting conditions, which has not previously been reported, and the second stage was to examine whether pramlintide could induce changes in plasma Aβ and other biomarkers to aid the diagnosis of AD in humans.

2. Methods

2.1. Participant recruitment

For the first study of this project, we recruited a group of 50 subjects from an existing registry at Boston University Alzheimer's Disease Center (BU ADC) to investigate the safety profile of pramlintide in the absence of diabetes and under fasting condition. The subjects in this pool had called to express interest in participating in AD research. The data core of BU ADC screened the potential participants who were aged 50 to 90 years and did not have diabetes, stroke, or history of brain injury. For the second study of this project, we used the subjects of the Healthy Outreach Program for the Elderly study [20].

The protocol and consent form were approved by the Institutional Review Board of Boston University School of Medicine. All enrolled participants provided informed consent. For the participants who carried the diagnosis of probable AD, each subject's next of kin also signed the informed consent.

2.2. Diagnoses of participants

These subjects have been followed annually for cognitive evaluation according to the National Alzheimer's Disease Coordinating Center protocol [21,22] and diagnosed at the consensus meetings at BU ADC. They all received a consensus diagnosis of normal cognition without memory complains (*n* = 8), or probable amnestic mild cognitive impairment (MCI; *n* = 7) or probable AD (*n* = 10). The diagnosis of dementia was based on *the Diagnostic and statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)* criteria. The the National Institute of Neurological and Communicative Disorder and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) guidelines [23,24] were used to determine whether the diagnostic criteria were met for possible or probable AD. The diagnostic criteria for MCI were based on the guidelines of Petersen et al. [25] and the updated criteria by Albert et al. [26]. Briefly, MCI diagnosis requires both reported and objective evidence of cognitive impairment, living independently, and the absence of dementia. Subjects were considered cognitively intact as control subjects if they were not demented and did not have MCI. For probable MCI, which is predicted to progress to AD, impairment in episodic memory was required. We then further divided the control subjects into those with and without self-complaint on memory.

2.3. Experimental protocol of the amylin challenge test

The entire study design is shown in Fig. 1. The study was conducted at the General Clinical Research Unit, Boston University Medical Center. Participants arrived in the morning after fasting for greater than 9 hours overnight. After obtaining written consent, subjects underwent baseline blood draws, vital sign checks, and blood glucose concentration determination, followed by intravenous line placement. If the blood glucose concentration was greater than 80 mg/dL, a subcutaneous injection of pramlintide, 60 μg, was administered, and blood draws were conducted at 5, 30, 60, and 180 minutes after the injection. The blood samples were centrifuged immediately after the blood draw. Plasma was isolated and stored at −80°C. At each time point, the glucose concentration and vital signs, including O_2 saturation, temperature, respiration rate, and heart rate, were monitored.

2.4. Measurements

To measure Aβ in plasma samples, the sandwich Aβ ELISA was used [27,28]. We used an enzyme-linked immunosorbent assay (ELISA) assay to measure amylin concentration in plasma according to the manufacturer's instructions (Cat: EZHA-52K; LINCO Research, St. Charles, MO). Single molecule array (Simoa) testing was
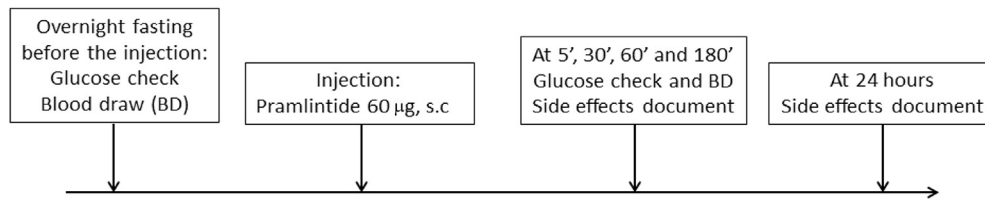
Fig. 1. Study design of the pramlintide challenge test. The diagram of the study design is shown. Human subjects without diabetes from BU ADC registry participated in the study with a single subcutaneous (s.c) of pramlintide and blood draws at different time points. Sixty minutes after the pramlintide challenge, the subjects had breakfast. Abbreviation: BU ADC, Boston University Alzheimer's Disease Center.

used to measure the tau protein in plasma samples (RayBiotech, Norcross, GA). Mulltiplex ELISA arrays were used to detect retinol-binding protein 4 (RBP-4) and interleukin 1 receptor antagonist (IL-1Ra) in plasma samples.

2.5. Statistical analysis

The statistical analysis was performed using SAS (version 9.1). All variables are shown as means ± standard error (SE). Mean group values were compared by two-way analysis of variance. Individual between-group comparisons were carried out by t tests. The a priori classification of the study subjects into three experimental groups (control subjects or MCI or AD) was used for the analyses. Each measurement of Aβ1–40, Aβ1–42, and t-tau in the pramlintide challenge test regardless of time points and participants was treated as a variable. Multivariate linear regression was used to examine the associations between each of Aβ1–40, Aβ1–42, or t-tau in the pramlintide challenge test as an outcome and the cognitive diagnoses while adjusting for age, gender, race, body mass index (BMI), apolipoprotein E (APOE) ε4 allele, and the time points of blood draws. For all analyses, the two-sided significance level of .05 was used.

Etiologic analysis and diagnosis (predictive) models were performed. We constructed and evaluated some key diagnostic models to differentiate individuals with MCI or AD from healthy control subjects based on logistic regression with cross-validations. The final predictive models were selected based on the smallest bootstrap prediction errors for the logistic regression models by using cross-validation (R Foundation for Statistical Computing, Vienna, Austria). We used ROCR [29] for evaluating and visualizing classifier performance and pROC [30] to calculate the area under the curve (AUC) of receiver operating characteristic curves of the final predictive models.

3. Results

3.1. Is pramlintide use safe in participants without diabetes under fasting condition?

Fifty subjects who did not have diabetes participated in the first part of the study (Table 1). The average age range was 71.5 ± 8.4 years. Twenty-seven subjects were females, and 23 were males. Six of the subjects were African Americans. The average BMI was 26.7 ± 4.4. Of the 38 subjects with APOE genotyping, 12 were APOE ε4 carriers.

Table 1 shows that in the pramlintide challenge test, none of the participants developed hypoglycemia defined by <70 mg/dL; four developed headaches, two had dizziness, and two had pruritus. All the symptoms were mild and transient that did not require treatment. Supplementary Table 1 shows that pramlintide mildly reduced the systolic blood pressure at 5 minutes and reduced the glucose concentration at 30 minutes. Food intake 1 hour after the pramlintide challenge increased blood glucose and heart rate and decreased blood pressure; these changes were expected and not clinically significant. These data indicate that a single injection of pramlintide was safe in the absence of diabetes under fasting conditions.

3.2. Comparisons of vital signs and pK levels among different diagnostic groups in the pramlintide challenge test

We further only used the participants who had a clear consensus diagnosis, as determined by the BU ADC, for the comparisons (Table 2). The AD group had the lowest Mini-Mental State Examination scores among the three diagnostic groups (Table 2). At baseline, the MCI group tended to have a lower concentration of plasma amylin than did the control or AD group, but the difference did not reach statistical significance (Supplementary Fig. 1A).

Table 1

Basal state of the study participants and their adverse events during the pramlintide challenge test

	$n = 50$
Basal state	
Age, y, mean ± SD	71.5 ± 8.4
Female/male, n	27/23
AA/Caucasian, n	6/44
APOE ε4 carrier, n/total	12/38
BMI, mean ± SD	26.7 ± 4.4
MMSE, mean ± SD	27.3 ± 4.3
Adverse events	
Hypoglycemia (<70 mg/dL), n/total	0/50
Nausea, n/total	0/50
Vomit, n/total	0/50
Abdominal pain, n/total	0/50
Headache, n/total	4/50
Dizziness, n/total	2/50
Pruritus, n/total	2/50

Abbreviations: AA, African Americans; APOE, apolipoprotein E; BMI, body mass index; MMSE, Mini-Mental State Examination; SD, standard deviation.

Table 2
Comparisons of demographics, cognition, and the AD biomarker changes induced by the pramlintide injection in control, MCI, and AD participants

Characteristics	Controls (N = 7)	MCI (N = 8)		AD (N = 10)	
	n (%) or mean ± SE*	n (%) or mean ± SE*	P value[†]	n (%) or mean ± SE*	P value[†]
Gender			.75		.04
Female	5 (71.4)	4 (50.0)		1 (10.0)	
Male	2 (28.6)	4 (50.0)		9 (90.0)	
Age (y)	71.9 ± 2.7	77.2 ± 2.5	.16	74.8 ± 2.2	.41
Race			1.00		1.00
Black	0 (0)	1 (12.5)		0 (10.0)	
White	7 (100)	7 (87.5)		10 (90.0)	
APOE ε4 carrier			.19		1.00
Yes	3 (42.9)	0 (0.0)		3 (42.9)	
No	4 (57.1)	7 (100.0)		4 (57.1)	
BMI (kg/m^2)	26.1 ± 2.0	28.1 ± 1.9	.46	26.7 ± 1.8	.82
MMSE (score)	29.4 ± 1.3	28.4 ± 1.2	.55	21.1 ± 1.2	.0001
Aβ1–40 (fold %)					
0 min	100.0 ± 0.0	100.0 ± 0.0	1.00	100.0 ± 0.0	1.00
5 min	87.5 ± 13.7	98.7 ± 12.8	.56	136.3 ± 11.5[a]	.01
30 min	84.8 ± 9.6	88.1 ± 9.0	.80	113.8 ± 8.0	.03
60 min	80.6 ± 12.4	92.1 ± 11.6	.50	115.9 ± 10.4	.04
180 min	73.8 ± 9.8	87.2 ± 9.2	.33	112.4 ± 8.2[a]	.006
Aβ1–42 (fold %)					
0 min	100.0 ± 0.0	100.0 ± 0.0	1.00	100.0 ± 0.0	1.00
5 min	103.0 ± 8.8	97.8 ± 8.2	.67	111.5 ± 7.3	.46
30 min	98.4 ± 8.8	93.8 ± 8.2	.70	109.7 ± 7.3	.33
60 min	92.3 ± 8.2	88.5 ± 7.6	.74	110.5 ± 6.8	.10
180 min	104.4 ± 9.6	90.2 ± 9.0	.29	110.2 ± 8.0	.64
T-tau (fold %)					
0 min	100.0 ± 0.0	100.0 ± 0.0	1.00	100.0 ± 0.0	1.00
5 min	98.1 ± 14.6	88.8 ± 13.4	.65	95.7 ± 11.6	.90
30 min	108.5 ± 18.9	119.6 ± 17.3	.67	83.5 ± 15.0	.32
60 min	111.2 ± 12.2	94.6 ± 11.1	.33	88.3 ± 9.6	.16
180 min	100.5 ± 13.2	91.7 ± 12.0	.63	85.1 ± 10.4	.37

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; BMI, body mass index; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; SE, standard error.

*Overall changes at all time points from the baseline and whether these changes differ between groups were analyzed. Aβ40 changes are shown significantly different from the baseline and the changes are also different between groups with [a]P value <.05.

[†]The control group is the reference group in the comparisons. The comparisons of count variables or the numbers at each time point are based on Fisher's exact test for small samples. The comparisons of continuous variables are based on analysis of variance to analyze the differences among group means (such as "variation" among and between groups) for three or more groups.

The peak of the drug concentration was reached at 30 minutes after the injection, and the pK levels of the drug in plasma were similar among all three diagnostic groups (Supplementary Fig. 1B). There were no differences in vital signs during the pramlintide challenge test among the three diagnostic groups (Supplementary Fig. 2).

3.3. Change in plasma Aβ in the pramlintide challenge test

At baseline, there was no statistical difference in the plasma Aβ1–40 levels among the three diagnostic groups (Fig. 2A). The pramlintide challenge increased the levels of total Aβ1–40 in plasma when compared with the baseline concentration only among those who carried the AD diagnosis at 5 minutes (P = .05), 180 minutes (P < .05), and tended to do so at other time points (Fig. 2B). More importantly, the pramlintide challenge on the Aβ1–40 levels in plasma separated AD subjects from control subjects at 5 (P = .01), 30 (P = .03), and 60 (P = .04) minutes after the

injection. After the 60 minutes point, the subjects were given breakfast and continued to show that Aβ1–40 levels were increased at 180 minutes point in the AD subjects when compared with control subjects (P = .006). The difference between the control and MCI groups did not reach statistical significance at each time point. As shown in Fig. 2C–E for individual cases, more subjects in the AD group, but not in the control and the MCI groups, had increased Aβ1–40 in plasma in the pramlintide challenge test. Four cases in the AD group had the evidence of AD pathology in the brain (Fig. 2E and Supplementary Table 2).

The AD group tended to have a higher level of plasma Aβ1–42 than the control group at baseline (P = .06) (Fig. 3A and Table 2). Although more individuals with a diagnosis of AD had an increase in plasma Aβ1–42 levels after the pramlintide challenge (Fig. 3E), as an AD group, the plasma Aβ1–42 level changes in the pramlintide challenge did not reach statistical significance when compared with the control group (Fig. 3B).
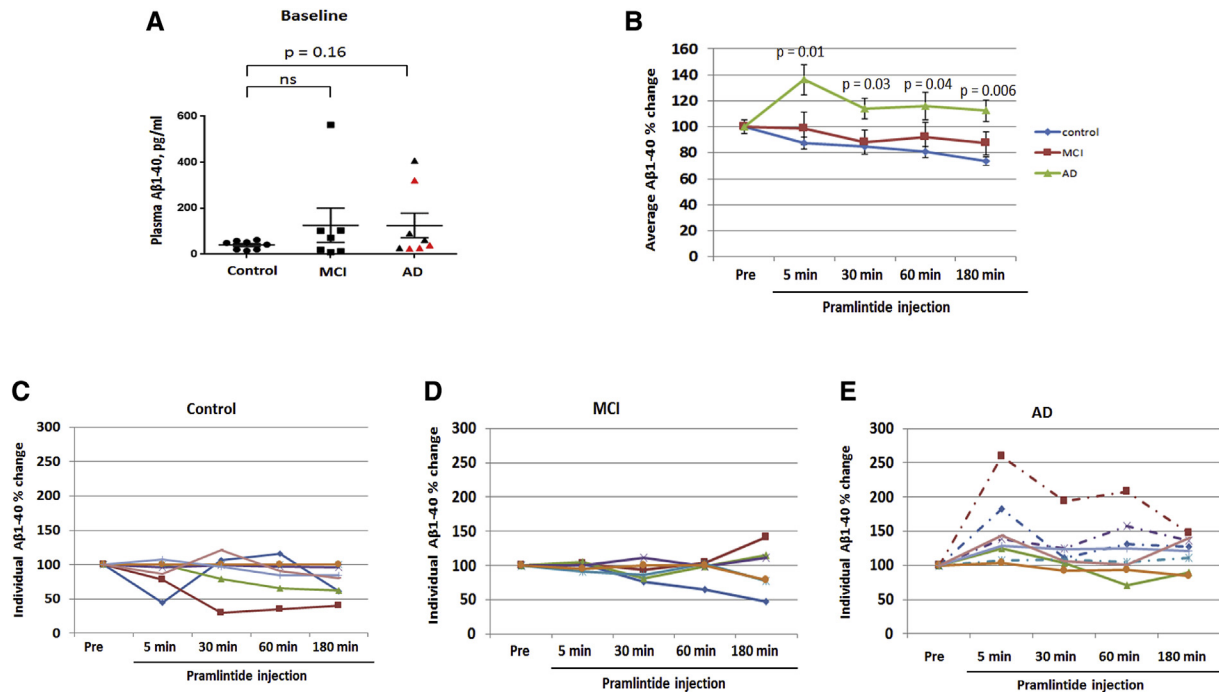
Fig. 2. Characterization of plasma Aβ1–40 change in the pramlintide challenge test. Scatterplots of preinjection plasma Aβ1–40 levels (A) and the average % changes (mean ± SE) in plasma Aβ1–40 from baseline at different time points before and after the injection of pramlintide (B) were examined for the control subjects, MCI, and AD. A *t* test was used to compare the control group and the MCI group or the AD group at each time point. Percent changes in plasma Aβ1–40 from the preinjection level after the injection of pramlintide at each time point for each individual participant were examined for the control (C), MCI (D), and AD (E) groups. The statistical significance with *P* values is shown for the comparisons between the control and AD groups at each time point. Red symbols and dashed lines represent the cases with the information on the AD pathology in the brain. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment.

3.4. Change in plasma total tau protein in the pramlintide challenge test

At baseline, the AD group tended to have higher levels of total tau (t-tau) in plasma than the control ($P = .12$) and MCI groups (Fig. 4A). When compared with the baseline concentration, the pramlintide challenge decreased t-tau levels in plasma at 30 minutes ($P = .02$), 60 minutes ($P = .08$), and 180 minutes ($P = .004$) only among those who carried AD diagnosis, but not in the control and MCI groups (Fig. 4B). However, unlike plasma Aβ1–40 in the pramlintide challenge test, the decrease in t-tau did not separate the AD group from the control group at each time point.

3.5. Multivariate regression analyses of the AD biomarkers in the pramlintide test

To further determine the effects of pramlintide challenge on the AD biomarker changes to diagnose AD, we treated each data at any time point (Table 2) as an individual variable and used multivariate linear regression analyses to examine the relationship between the biomarker changes as an outcome and the diagnoses, MCI and AD, as the determining factors after adjusting for confounders (Table 3). AD (β = +37.40, SE = 6.84, $P < .0001$) and MCI (β = +17.97, SE = 6.95, $P < .01$) were positively associated with plasma Aβ1–40 after adjusting for age,

gender, *APOE* ε4 allele, BMI, and different time points. AD (β = +12.76, SE = 5.15, $P = .01$) was positively associated with plasma Aβ1–42 in the same model, whereas MCI (β = −11.52, SE = 5.23, $P = .03$) was negatively associated with plasma Aβ1–42 in the same model. AD, but not MCI, was negatively associated with plasma t-tau (β = −22.85, SE = 8.23, $P = .007$) after adjusting for confounders. In addition, only age was negatively associated with plasma Aβ1–40, whereas BMI was positively associated with plasma Aβ1–42 significantly in the regression analyses. Taken together, the pramlintide challenge provoked a surge of plasma Aβ and a decrease in plasma t-tau only in AD, indicating a value of this test for diagnosing the disease.

3.6. Change in plasma metabolic and inflammatory factors in the pramlintide challenge test

We next explored the metabolic and inflammatory biomarkers in the pramlintide challenge test. A single injection of pramlintide tended to increase the RBP-4 level in the control group but decreased RBP-4 level in the AD and MCI groups, although statistical significance was not reached. However, at 60 minutes after the pramlintide challenge, but not at baseline, the result of RBP-4 levels separate AD and control groups ($P = .01$) (Fig. 5A). The difference in
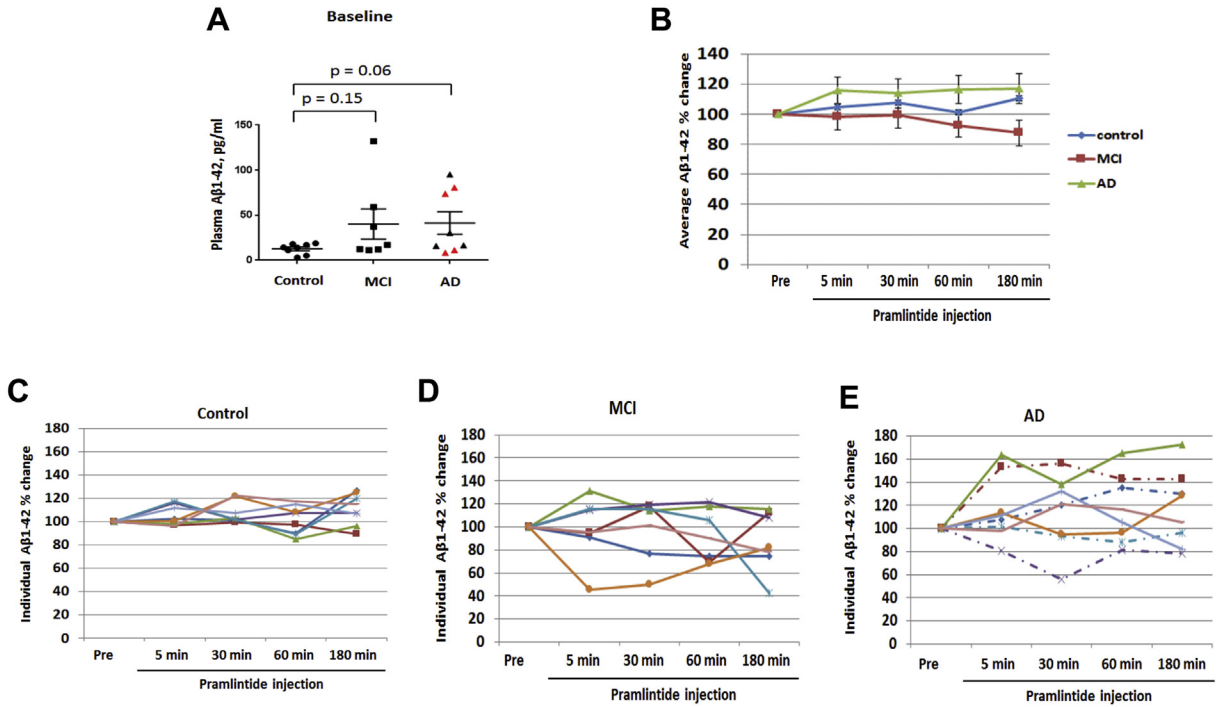
Fig. 3. Characterization of plasma Aβ1–42 change in the pramlintide challenge test. Scatterplots of preinjection plasma Aβ1–42 levels (A) and the average % changes (mean ± SE) in plasma Aβ1–42 from the baseline at different time points before and after the injection of pramlintide (B) were examined for the control subjects, MCI, and AD. A *t* test was used to compare the control group and the MCI group or the AD group at each time point. Percent changes in plasma Aβ1–42 from the preinjection level after the injection of pramlintide at each time point for each individual patient were examined for the control (C), MCI (D), and AD (E) groups. The statistical significance with *P* values is shown for the baseline comparisons between the control and MCI or AD groups. Red symbols and dashed lines represent the cases with the information on the AD pathology in the brain. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; SE, standard error.

plasma RBP-4 between the control group and the MCI group only showed this trend. We found that at 60 minutes after the pramlintide injection, but not at baseline, the average concentrations of IL-1Ra in the AD subjects were higher than in those with normal cognition (*P* = .04) (Fig. 5B). Because RBP-4 is also related to inflammation [31], we found that the ratio of IL-1Ra/RBP-4 was also higher in the AD group than the control group (*P* = .03) (Fig. 5C). Although the majority in the control and MCI groups decreased the levels of IL-1Ra/RBP-4 ratio, six of 10 cases in the AD group had an increased level of IL-1Ra/RBP-4 ratio by the pramlintide challenge (Fig. 5D).
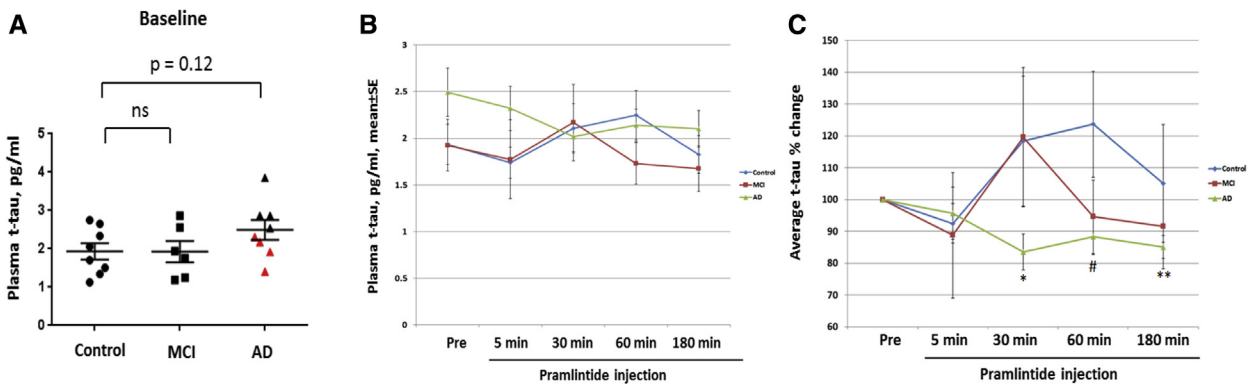

Fig. 4. Characterization of plasma tau change in the pramlintide challenge test. Scatterplots of preinjection plasma tau levels (A) and the average changes (mean ± SD) in plasma tau (B) and the average % changes from baseline (C) were examined for the control subjects, MCI, and AD at different time points before and after the injection of pramlintide. A *t* test was used to compare the levels between the control group and the MCI group or the AD group at each time point and did not show differences (B and C). However, the statistical significance with *P* values is shown for the comparisons between the baseline and the average fold change at each time point for the AD but not for the control and MCI groups, *P* = .02; **P* = .004; and #*P* < .10 (C). Red symbols represent the cases with the information on the AD pathology in the brain. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; SD, standard deviation.

Table 3
The effects of pramlintide challenge test on the AD biomarkers in multivariate regression analyses

Changes	Plasma Aβ1–40		Plasma Aβ1–42		Plasma t-tau	
	Estimate β (SE)	*P* value	Estimate β (SE)	*P* value	Estimate β (SE)	*P* value
Age, y	−1.16 (0.50)	.02	−0.30 (0.38)	.42	+0.23 (0.64)	.75
Male	+13.58 (6.40)	.04	−1.75 (4.81)	.72	+15.65 (8.20)	.06
Caucasian	−16.82 (14.26)	.24	−10.83 (10.72)	.31	+21.67 (16.45)	.19
APOE ε4 carrier	+9.30 (6.22)	.14	−6.38 (4.67)	.17	+5.34 (7.68)	.49
BMI	−0.12 (0.37)	.74	+0.86 (0.28)	.003	−0.18 (0.44)	.63
Postinjection 5′	+10.57 (7.17)	.14	+4.72 (5.39)	.38	−5.83 (9.20)	.53
Postinjection 30′	−2.54 (7.17)	.72	+1.41 (5.39)	.79	+1.49 (9.20)	.87
Postinjection 60′	−1.63 (7.17)	.82	−1.61 (5.39)	.77	−3.67 (9.20)	.69
Postinjection 180′	−6.49 (7.17)	.37	+2.18 (5.39)	.69	−8.77 (9.20)	.34
MCI	+17.97 (6.95)	.01	−11.52 (5.16)	.03	−2.68 (9.23)	.77
AD	+37.40 (6.84)	<.0001	+12.52 (5.23)	.01	−22.85 (8.23)	.007

Abbreviations: AD, Alzheimer's disease; BMI, body mass index; MCI, mild cognitive impairment; SE, standard error.
NOTE. All participants with diagnoses and multivariate analyses were used with the fold changes in each biomarker at all time points as an outcome and with MCI and AD as determining factors in each model. The confounders were adjusted for each model as described in the table.

3.7. Can the pramlintide challenge test be used to diagnose AD?

Given the result of mechanistic study that amylin removes Aβ out of the AD brain in AD mouse models [17], we conducted etiologic analysis and diagnosis (predictive) models to evaluate Aβ1–40 and Aβ1–42 in the pramlintide challenge test in humans. The predictive model for AD versus control subjects included the general confounding factors (gender, age, and *APOE* ε4), fold changes in Aβ1–40 (odds ratio [OR] = 4.90; 95% confidence interval [CI] = 1.67, 3.77; *P* = .03), and Aβ1–42 (OR = 6.52; 95% CI = 1.87, 5.57; *P* = .02). The predictive model for MCI versus control subjects included the same general confounding factors: fold changes in Aβ1–40 (OR = 2.21; 95% CI = 1.23, 4.56; *P* = .02) and Aβ1–42 (not significant). The
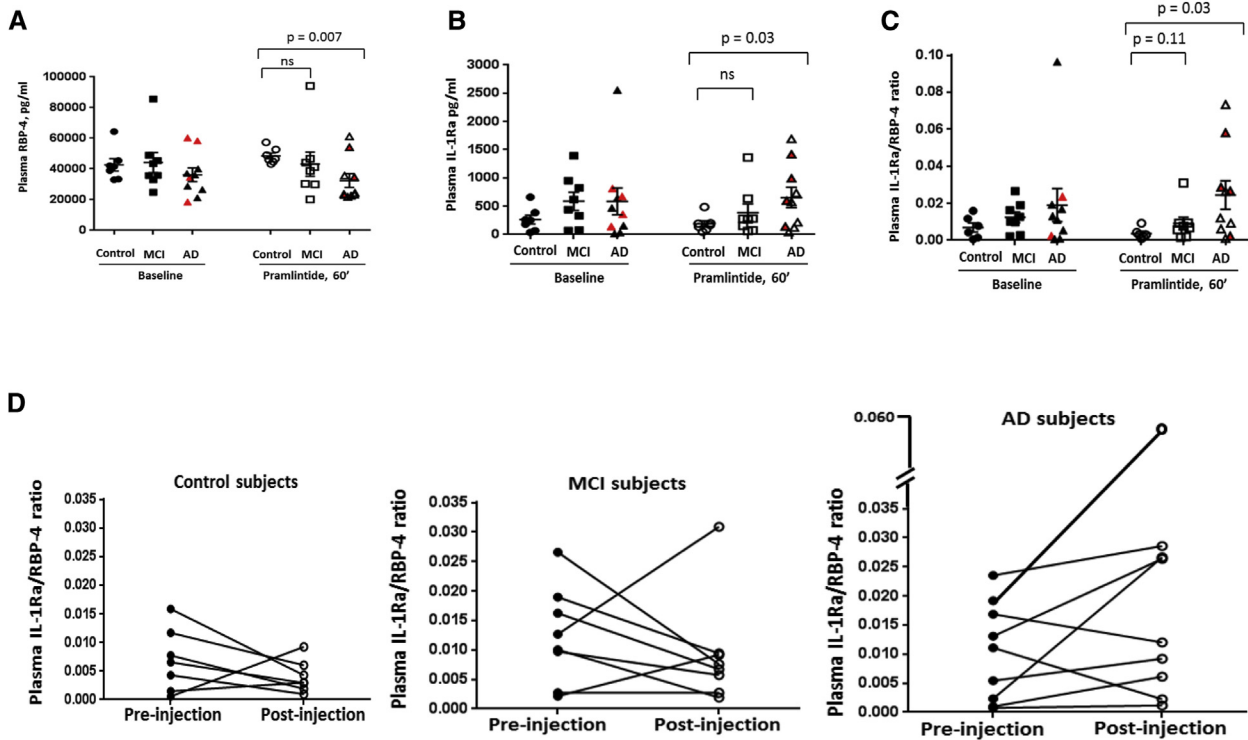

Fig. 5. Characterization of metabolic and proinflammatory changes in the pramlintide challenge test. Scatterplots of plasma RBP-4 (A), IL-1Ra (B), the ratio of IL-1Ra/RBP-4 (C), and individual IL-1Ra/RBP-4 in each diagnostic group (D) at the time points before injection and 60 minutes after injection were examined for the control subjects, MCI, and AD. A *t* test was used to compare the control group and the MCI group or the AD group at each time point, and the statistical significance with *P* values is shown. Red symbols represent the cases with the information on the AD pathology in the brain. Abbreviations: AD, Alzheimer's disease; IL-1Ra, interleukin 1 receptor antagonist; RBP-4, retinol-binding protein 4.

AUC for AD's predictive model was 0.986 (95% CI = 0.965, 1) (Fig. 6A) and the AUC for MCI's predictive model was 0.87 (95% CI = 0.78, 0.96) (Fig. 6B), respectively.

In the AD group, we had one subject (ID 1002) who passed away after the pramlintide challenge test, and his autopsy found that he had both pathologies of AD and lewy body disease in his brain (Supplementary Table 2). There were four cases, including the subject 1002 who had an AV-45 PET scan, which showed positive amyloid imaging and had fluorodeoxy glucose (FDG) positron emission tomography (PET) scans showing hypometabolism in their brains. We explored using the combination score of different biomarkers tested in the pramlintide challenge test. We found that the AD group had a significantly higher average combined score (3.63 ± 1.06 vs. 0.17 ± 0.41, $P = .00001$) and the MCI group (1.57 ± 0.96 vs. 0.17 ± 0.41, $P = .008$) than the control group (Supplementary Table 2). Compared with the MCI group, the AD group had a higher score ($P = .002$).

4. Discussion

Pramlintide is a human amylin analog substituting prolines at positions 25, 28, and 29, which prevent amylin from oligomerizing or aggregating [32], and has become an Food and Drug Administration-approved drug for diabetes. Recently, using AD mouse models our study and others demonstrate that amylin-type peptides have potential therapeutic and diagnostic benefits for AD [17,18]. This study translated the mouse findings into humans and laid some foundation to repurpose pramlintide for AD. Combining the data from AD mouse models and humans, our study suggests a potential of amylin-type peptides for AD diagnosis and therapeutics.

The major adverse event of pramlintide is hypoglycemia and the most common adverse effect of it is nausea when treating diabetes [33–35]. It is thus critical to demonstrate the safety profile of the drug for patients with AD, who often do not have diabetes but have poor appetite and food intake. This study showed that a single injection of pramlintide to nondiabetic human subjects did not induce hypoglycemia and nausea even under fasting conditions (Table 1). Other adverse effects of pramlintide seen in diabetes were also mild and transient in nondiabetic subjects. Pramlintide use for diabetes in clinic is generally safe, and the occurrence of hypoglycemia is rare and often when combined with insulin treatment [19]. Diabetic patients with a long duration of the disease are more likely to have reduced secretion of amylin [36] and diabetes increases the risk factor for developing AD [37,38]. Thus, pramlintide will probably be safe and beneficial to be repurposed for AD regardless diabetes status.

Our study suggests a potential role for the creation of a blood test that relies on the pramlintide challenge, which could cross the BBB and help to translocate the biomarkers related to AD pathology including Aβ and neuroinflammation, from the brain into the bloodstream where they can be detected. An increase in Aβ1–40 induced by the pramlintide test significantly separated AD from control subjects and MCI; therefore, this test has potential for AD diagnosis (Figs. 2, 3, and 6). Currently, the diagnostic tests by using blood Aβ have been proved not valid for AD diagnosis [4–8]. One possibility is that the existence of the BBB and blood-CSF barrier makes it difficult to detect brain-originating biomarkers in the blood [39,40]. Using AD mouse models, we also found that a single peripheral injection of amylin or pramlintide also induces a surge of Ab in the blood and the blood surge of Ab correlates with the amyloid pathology in the brain [17]. Therefore, the increase in blood Aβ in the pramlintide challenge test was likely from the translocation of Aβ from the AD brain in humans.
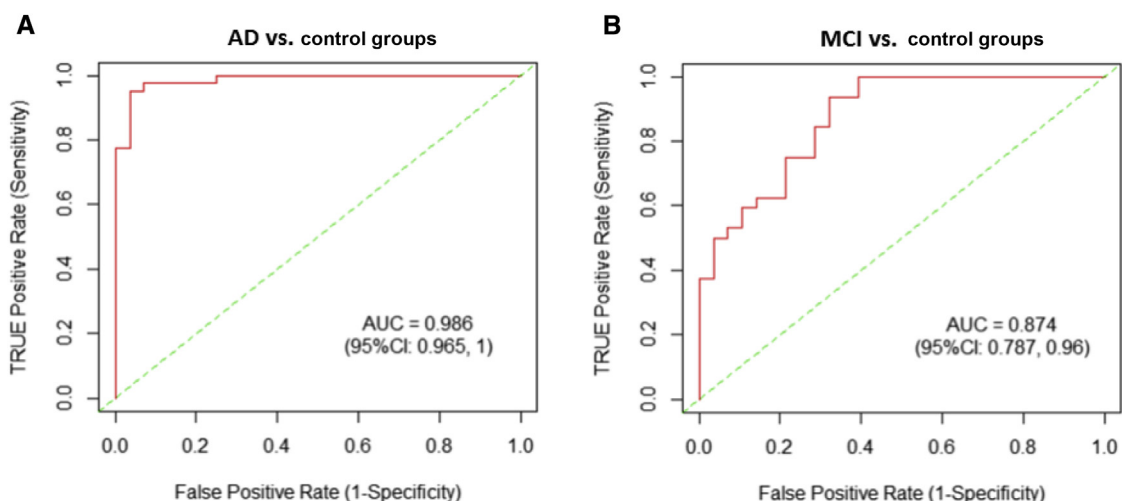

Fig. 6. ROC curve analysis of the Aβ changes in the pramlintide challenge test. Etiologic analysis and diagnosis (predictive) models were used to evaluate the fold changes in both Aβ1–40 and Aβ1–42 in the predictions of AD and MCI. The area under the curve (AUC) of ROC curves of the final predictive models for AD versus control subjects (A) and for MCI versus control subjects (B) are shown. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; ROC, receiver operating characteristic.

Amylin shares its secondary structure with both Aβ and part of the tau protein [41,42]. At baseline the plasma t-tau protein level only tended to be higher in AD subjects (Fig. 4A), which is consistent with previous studies [43] but cannot be used for the diagnosis. On the other hand, the pramlintide challenge test significantly induced a decrease in plasma t-tau only in AD but not in control subjects and MCI (Fig. 4B and C). Although the mechanism is unclear, the t-tau changes in the pramlintide challenge test could be another useful biomarker for AD.

The pramlintide challenge tended to decrease the blood level of RBP-4 in AD but probably increased the level in the subjects with normal cognition (Fig. 5A). Because RBP-4 is linked with insulin resistance in the brain and is increased in the brain of AD mouse models [31], these data might reveal the AD pathology in the brain as the change in RBP-4 could be a useful biomarker for AD. As neuroinflammation is a key, but not specific, component of AD pathology in the brain, especially at a late stage of the disease [44,45], our findings showed that the pramlintide challenge increased IL-1Ra in AD but not in MCI (Fig. 5). A previous study showed that approximately 200 proteins, most of which are related to inflammation, were measured in plasma samples to identify a protein signature associated with patients who progressed from MCI to the dementia stage of AD [46]. Because that amylin passes through the BBB easily [12,13] and that amylin modifies inflammation [14], these biomarker changes in the pramlintide challenge test might also directly or indirectly reveal the brain pathology.

AD has a chronic and long neurodegenerative process, and using a single blood biomarker to diagnose AD might not be valid for revealing the brain pathology for the whole pathologic cascade. For example, at an early stage, more Aβ is soluble in the brain, so it is more likely to be released into blood; at a late stage, more Aβ is aggregated in plaques, so it is less likely to be released into blood. As the disease progresses, there are worsening proinflammatory reactions. Our study showed the potential to use the pramlintide challenge to mobilize the AD biomarkers from the brain into the blood and to use the combined signature of the AD biomarkers for the diagnosis (Supplementary Table 2). It is shown that amylin binds to amylin receptor to relax cerebral arteries and increase local cerebral blood flow [16,47]. This could be the mechanism that the pramlintide challenge dilates cerebral blood vessels to remove the toxic peptides including Aβ and inflammation factors from the brain into the blood.

The limitation of this pilot study was the relatively small number in each diagnostic group and only a few of the AD subjects had brain imaging or autopsy data available to confirm AD pathology in the brain. In addition, we did not have the data to compare the results of the pramlintide challenge test between those subjects with positive and with negative brain imaging for AD. Because our study only observed a trend of Aβ1–42 in the pramlintide challenge

test with a single dose (Fig. 3), higher doses of pramlintide may be necessary for the test to detect Aβ1–42. Nevertheless, we provided the evidence for the concept of a probable challenge test to diagnose AD, which is analogous to the oral glucose tolerance used in diabetes diagnosis, with patients showing abnormalities of glucose metabolism after a glucose challenge. If established, the test would be simple and specific and could be easily performed in a doctor's office to diagnose AD.

Acknowledgments

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trci.2016.12.002.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using the sources of PubMed and Google search. While aggregation property of amylin and the toxicity of aggregated amylin for neuronal cells have been widely studied, amylin as an important gut-brain hormone is less studied. There have been two recent publications including our own used AD mouse models to demonstrate that amylin and its clinical analog, pramlintide, have a potential for Alzheimer's disease (AD) diagnosis and treatment. These relevant citations are appropriately cited.

2. Interpretation: Our findings were the first to show that pramlintide use appeared to be safe in the absence of diabetes in humans. The biomarker changes as a result of a single injection of pramlintide which distinguished AD from controls and mild cognitive impairment (MCI). This is consistent with the preclinical findings.

3. Future directions: The manuscript proposes a framework for the repurpose of pramlintide for AD and the conduct of additional human studies. Examples include: (a) further establishment of the pramlintide challenge test for AD and MCI; (b) correlation between the result of pramlintide challenge test and the AD biomarkers in central nervous system (CNS); (c) examining the safety of pramlintide for AD in phase I clinical trial.

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