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Fibrinogen-Derived $\gamma^{377\text{-}395}$ Peptide Improves Cognitive Performance and Reduces Amyloid-β Deposition, without Altering Inflammation, in AβPP/PS1 Mice

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Abstract. Fibrinogen has emerged as a promising therapeutic target against Alzheimer's disease because of its dual role in altered vascular function and amyloid-β aggregation. Here we provide evidence regarding cognitive improvement and reduction of brain parenchyma amyloid-β deposition in AβPP/PS1 mice after treatment for one month with the fibrinogen-blocking peptide Fib$\gamma^{377-395}$. No alteration in glial response or other neuroinflammatory markers was observed in the cortex of treated animals. Considering these results and the fact that Fib$\gamma^{377-395}$ does not affect coagulation function, this peptide could be considered as a promising and safe candidate for chronic treatment of Alzheimer's disease.

Keywords: AβPP/PS1 mice, Alzheimer's disease, amyloid, fibrinogen, inflammation

INTRODUCTION

Previous findings have demonstrated that the clotting protein fibrinogen may play a relevant role in Alzheimer's disease (AD) by linking altered vascular function and amyloid-β (Aβ) aggregation [1]. AD and related animal models exhibit altered blood-brain barrier permeability, decreased blood-flow, and increased susceptibility to ischemia, thus supporting the vascular pathology as a relevant factor in AD [2]. Fibrinogen is not normally found in brain but blood-brain barrier dysfunction caused by Aβ vascular accumulation, inflammatory processes, and ischemic episodes likely facilitates fibrinogen extravasation in the brain parenchyma. Extravascular fibrinogen potentiates, in turn, blood-brain barrier permeability by decreasing the levels of endothelial tight junction proteins. More importantly, extravascular fibrinogen can interact and co-deposit with Aβ, enhancing Aβ fibrillization and fibrinogen oligomerization in cerebral vessel walls and brain parenchyma leading to blood vessel occlusion, hypoperfusion, synaptic dysfunction, and neuronal degeneration [3–5]. Moreover, fibrinogen

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released in the brain parenchyma promotes neuroinflammatory responses [6], which have deleterious effects when sustained chronically [7].

In light of these observations, blocking the activity of fibrinogen has emerged as a putative therapeutic action to reduce vascular and Aβ pathologies in AD. In this line, genetic depletion of fibrinogen decreases cerebral amyloid angiopathy and reduces cognitive impairment in a transgenic model of AD [4]. Similarly, the use of anticoagulant agents that remove fibrinogen from circulation is also beneficial in AD models, in terms of reduced neurovascular damage and neuroinflammation [4, 6]. However, their practical use in AD is limited because of the side effects related to coagulation deficiency derived from their chronic administration, particularly in the elderly [8].

The aim of the present study was to test the therapeutic properties of the fibrinogen-derived $\gamma^{377-395}$ peptide (Fib$\gamma^{377-395}$) in an animal model of AD. This peptide is known to inhibit the interaction between fibrinogen and the microglia integrin receptor Mac-1 (CD11b/αMβ2), resulting in an anti-inflammatory effect in at least two animal models of degenerative diseases, multiple sclerosis [9] and Duchenne muscular dystrophy [10, 11], without impairing fibrinogen's coagulation function. We have chosen the AβPP/PS1 mouse as a disease model because it replicates relevant features of AD including cognitive impairment, Aβ deposition, and neuroinflammation [12, 13].

MATERIALS AND METHODS

Animals

Experiments were carried out on male AβPP/PS1 mice and wild-type (WT) littermates aged 6 months (early symptomatic phase) at the onset of the study. The generation of mice expressing the human mutated forms AβPPswe and PS1dE9 (AβPP/PS1) has already been described [12]. Animals were maintained under standard housing conditions in a 12-h dark-light cycle with free access to food and water. Mice were randomly assigned to treatment groups (WT Vehicle: $n=7$; WT Fib$\gamma^{377-395}$: $n=8$; AβPP/PS1 Vehicle: $n=6$; AβPP/PS1 Fib$\gamma^{377-395}$: $n=7$) and the experiments were conducted under blind experimental conditions. All animal procedures were carried out following the guidelines of the European Communities Council Directive 2010/63/EU and with the approval of the local ethical committees of the University of Barcelona.

Pharmacological treatment

Fibrinogen $\gamma^{377-395}$ peptide (Y S M K E T T M K I I P F N R L S I G; Azco Pharmchem, San Jose, CA, US) at a concentration of 3 mg/mL or the corresponding vehicle (0.9% NaCl) was administered intranasally (5 μL) to mice every other day for 4 weeks, as previously described [9].

Behavioral evaluation of memory performance and sample collection

After a wash-out period (3 days), the memory performance of the animals was evaluated in the two-object recognition test, by using a V-maze as previously described [13]. On day 1, mice were habituated for 9 min allowing them to freely explore the apparatus. On the second day, mice were placed for 9 min in the maze where two identical objects were situated at the end of the arms, and the time that each mouse spent exploring each object was recorded. Then, 24 h after the training session, animals were placed again in the V-maze where one of the two familiar objects was replaced by a novel object. The time that the animals spent exploring the two objects was recorded and an object recognition index was calculated as the difference between the time spent exploring the novel and the familiar object, divided by the total time spent exploring the two objects. Animals with memory impairment revealed a lower object recognition index. At the end of the behavioral testing, the animals were killed and their brains rapidly removed from the skull and processed for study. One hemisphere was dissected on ice, immediately frozen, and stored at −80°C until used for the gene expression study. The other hemisphere was fixed in 4% paraformaldehyde and processed for immunohistochemistry.

Aβ and fibrinogen immunohistochemistry

Fixed tissue samples were embedded in paraffin, and coronal sections, 4 μm thick, were cut with a microtome. De-waxed sections were incubated with 98% formic acid (3 min, only for Aβ immunostaining) and then treated with citrate buffer (20 min) to enhance antigenicity. Then endogenous peroxidases were blocked by incubation in 10% methanol-1% H_2O_2 solution (15 min). Sections were blocked with 3% normal horse serum solution and then incubated at 4°C overnight with the primary antibody against total Aβ (clone 6F/3D, mouse, 1:50, Dako, Glostrup, Denmark) or fibrinogen (rabbit, 1:100, Abcam, UK).

Sections were subsequently rinsed and incubated with biotinylated secondary antibody (Dako), followed by EnVision+ system peroxidase (Dako), and finally with chromogen diaminobenzidine and H_2O_2. Sections were lightly counterstained with hematoxylin. After staining, the sections were dehydrated and cover-slipped for observation under a Nikon Eclipse E800 microscope (Nikon Imaging Inc., Tokyo, Japan; Objective: 10×). The cortical total Aβ burden was calculated as the percentage of the area of amyloid deposition in plaques with respect to the total area in 9 representative pictures taken of the cerebral cortex of each animal, corresponding to the main regions where Aβ deposition is observed in AβPP/PS1 mice. Aβ quantification was calculated using the Adobe® Photoshop® CS4 software (Adobe Systems Inc., San Jose, CA, USA), as previously described [13]. The vascular Aβ and fibrinogen deposition was also quantified as the percentage of vessels presenting Aβ- or fibrinogen-specific staining in a given brain section. Vessels were identified by their morphology based on hematoxylin staining.

Aβ soluble quantification: Enzyme-linked immunosorbent assay (ELISA) and dot blot assay

Fresh-frozen mouse brain cortex was homogenized in 4 volumes (wt:vol) of TBS extraction buffer (140 mM NaCl, 3 mM KCl, 25 mM Tris (pH 7.4), 5 mM EDTA, and protease inhibitor cocktail (Roche Molecular Systems, Pleasanton, CA, USA)). Homogenate was spun 100,000 g × 1 h, and the supernatant was saved as the soluble fraction for Aβ quantification. $A\beta_{40}$ and $A\beta_{42}$ Human ELISA kits (Invitrogen™ Corporation, Camarillo, CA, USA) were used to quantify the levels of $A\beta_{40}$ and $A\beta_{42}$ peptides in the brain soluble fractions. Quantitative determination was carried out according to the manufacturer's instructions, as previously described [13]. $A\beta_{40}$ and $A\beta_{42}$ levels were normalized to the total amount of protein from each individual sample (BCA method, Thermo Fisher Scientific, Wilmington, DE, USA).

Ten micrograms of each soluble sample was applied to nitrocellulose membranes using a 48-well Dot Blot Manifold (Cleaver Scientific, Rugby, United Kingdom). The membranes were blocked with 10% nonfat milk in TBS containing 0.01% Tween 20 at room temperature for 1 h. The membranes were washed 3 times, for 5 min each, with TBS containing 0.01% Tween 20 and incubated for 2 h at room temperature with rabbit polyclonal anti-prefibrillar oligomer antibody A11 (1:1000; Invitrogen Thermo-Fisher, Carlsbad, CA) or with rabbit polyclonal anti-fibrillar oligomer antibody OC (1:5000; Merck Millipore, Billerica, MA, USA). After washing, the membranes were incubated for 1 h with the appropriate horseradish peroxidase conjugated secondary antibody (1:2000; Dako), and immune complexes were visualized with a chemiluminescence reagent (electrochemiluminescence; Amersham). Densitometric quantification was carried out with TotalLab v2.01 software (Pharmacia, Sweden). Bands were normalized to the densitometric mean values of vehicle-treated AβPP/PS1 mice.

Double-labeling immunofluorescence

De-waxed sections were incubated with 98% formic acid (3 min) for Aβ immunofluorescence and then treated with citrate buffer (20 min) to enhance antigenicity. Sections were stained with a saturated solution of Sudan black B for 30 min (Merck Millipore) to block lipofuscin autofluorescence, then rinsed in 70% ethanol and washed in distilled water. After a blockade with 10% fetal bovine serum (90 min), the sections were incubated at 4°C overnight with combinations of primary antibodies against Aβ (clone 6F/3D, mouse, 1:50, Dako), glial fibrillary acidic protein (GFAP; rabbit, 1:250, Dako), IBA1 (rabbit, 1:250, Wako, Richmond, VA, USA) or fibrinogen (rabbit, 1:100, Abcam). After washing, the sections were incubated with Alexa488 or Alexa546 fluorescence secondary antibodies against the corresponding host species (1:400, Molecular Probes, Eugene, OR, USA). Then they were washed and mounted in Immuno-Fluore Mounting medium (ICN Biomedicals, Solon, OH, USA), sealed, dried overnight, and examined with a Nikon Eclipse E800 microscope. The specific GFAP, IBA1, and fibrinogen immunostaining density was calculated in reference to the Aβ plaque area in five representative pictures taken of the cortex of each animal using the Adobe® Photoshop® CS4 software.

Quantitative PCR

1 μg total RNA was reverse-transcribed with cDNA synthesized with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantification of the mRNA levels was performed in duplicate reactions with gene-specific TaqMan® probes and the TaqMan® Universal PCR Master Mix (Applied Biosystems). House-keeping genes used were Aars, Hprt, and Xpnpep1 [14]. QPCR was performed using the Applied Biosystems 7900HT Fast Real-Time PCR System. Samples were analyzed with the double delta CT (ΔΔCT) method using vehicle-treated wild-type

samples as control. Genes analyzed and probes are shown in Supplementary Table 1.

Statistical analysis

Behavioral and gene expression data were analyzed with two-way ANOVA with genotype and treatment as between factors, followed by Bonferroni's *post hoc* test for multiple comparisons when required. Amyloid and glial quantifications were analyzed with Student's *t*-test. In all the experiments, the significance level was set at $p < 0.05$.

RESULTS

Fib$\gamma^{377-395}$ peptide reduced cognitive deficits in AβPP/PS1 mice

Intranasal administration of the Fib$\gamma^{377-395}$ peptide for 4 weeks at the early phase of the symptomatic stage (6 months) reversed memory impairment of AβPP/PS1 mice on the two-object recognition test (Fig. 1). Two-way ANOVA revealed a significant treatment effect ($F_{(1,19)} = 8.493$, $p < 0.01$) and interaction between genotype and treatment factors ($F_{(1,19)} = 8.784$, $p < 0.01$). Subsequent *post hoc* comparisons revealed memory impairment in vehicle-treated AβPP/PS1 mice with respect to wild-type littermates ($p < 0.01$) and a significant memory improvement in the Fib$\gamma^{377-395}$ peptide-treated AβPP/PS1 when compared to vehicle ($p < 0.01$). Total exploration time on the training and test days is included in Table 1, showing no significant effect of genotype or treatment in the exploratory behavior.

Aβ cortical was reduced in AβPP/PS1 mice treated with Fib $\gamma^{377-395}$ but no modification was observed on Aβ vascular deposition and Aβ soluble contents

The cortical Aβ burden was significantly reduced in Fib$\gamma^{377-395}$ peptide-treated mice when compared to vehicle-treated AβPP/PS1 mice ($p < 0.05$, Fig. 2A, B). In contrast, the percentage of vessels showing Aβ deposition (Vehicle: $22.06 \pm 3.74\%$; Fib$\gamma^{377-395}$: $18.65 \pm 3.41\%$; Fig. 2B) or $A\beta_{40}$ and $A\beta_{42}$ soluble cortical contents were not significantly modified by Fib$\gamma^{377-395}$ peptide treatment (Fig. 2C). Similarly, no difference was observed on the prefibrillar (A11 antibody) or fibrillar oligomer (OC antibody) contents in soluble fractions of treated AβPP/PS1 mice evaluated by dot blot (Fig. 2D).

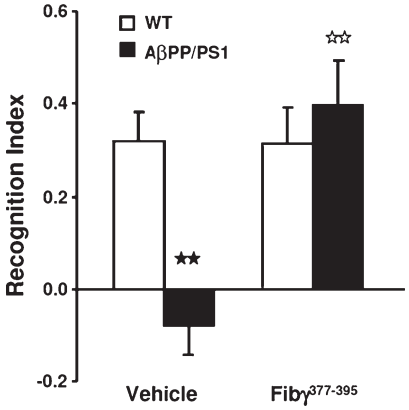

Fig. 1. Memory performance of animals treated during the early symptomatic stage (6 months). AβPP/PS1 mice chronically treated with vehicle exhibit a significant reduction in the recognition index when compared to corresponding wild-type littermates. In contrast, chronic administration of Fib$\gamma^{377-395}$ peptide induces memory improvement in AβPP/PS1 compared to vehicle-treated mice. Data are expressed as the mean values ± SEM. ★★$p < 0.01$ genotype effect; ☆☆$p < 0.01$, compared to vehicle.

Fib $\gamma^{377-395}$ reversed the increase in fibrinogen vascular deposition of AβPP/PS1 mice but did not modify fibrinogen contents in Aβ plaques

The percentage of vessels showing fibrinogen deposition was analyzed in treated mice. Two-way ANOVA revealed a significant genotype effect ($F_{(1,12)} = 10.820$, $p < 0.01$) and interaction between genotype and treatment factors ($F_{(1,12)} = 2.918$, $p < 0.01$). Subsequent

Table 1
Total exploration time on the two-object recognition test

		Exploration time on test day (s)			Exploration time on training day (s)								
					Familiar object			Novel			Total		
Treatment	Genotype	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Vehicle	Wild-type	30.97	±	6.97	6.49	±	1.43	10.61	±	1.96	17.10	±	2.56
	AβPP/PS1	21.48	±	4.16	6.62	±	1.54	5.60	±	1.55	12.21	±	3.06
Fibg$^{355-377}$	Wild-type	23.81	±	6.54	5.48	±	1.94	12.80	±	4.87	18.28	±	6.73
	AβPP/PS1	20.17	±	5.12	4.52	±	1.69	11.90	±	3.58	16.42	±	5.13

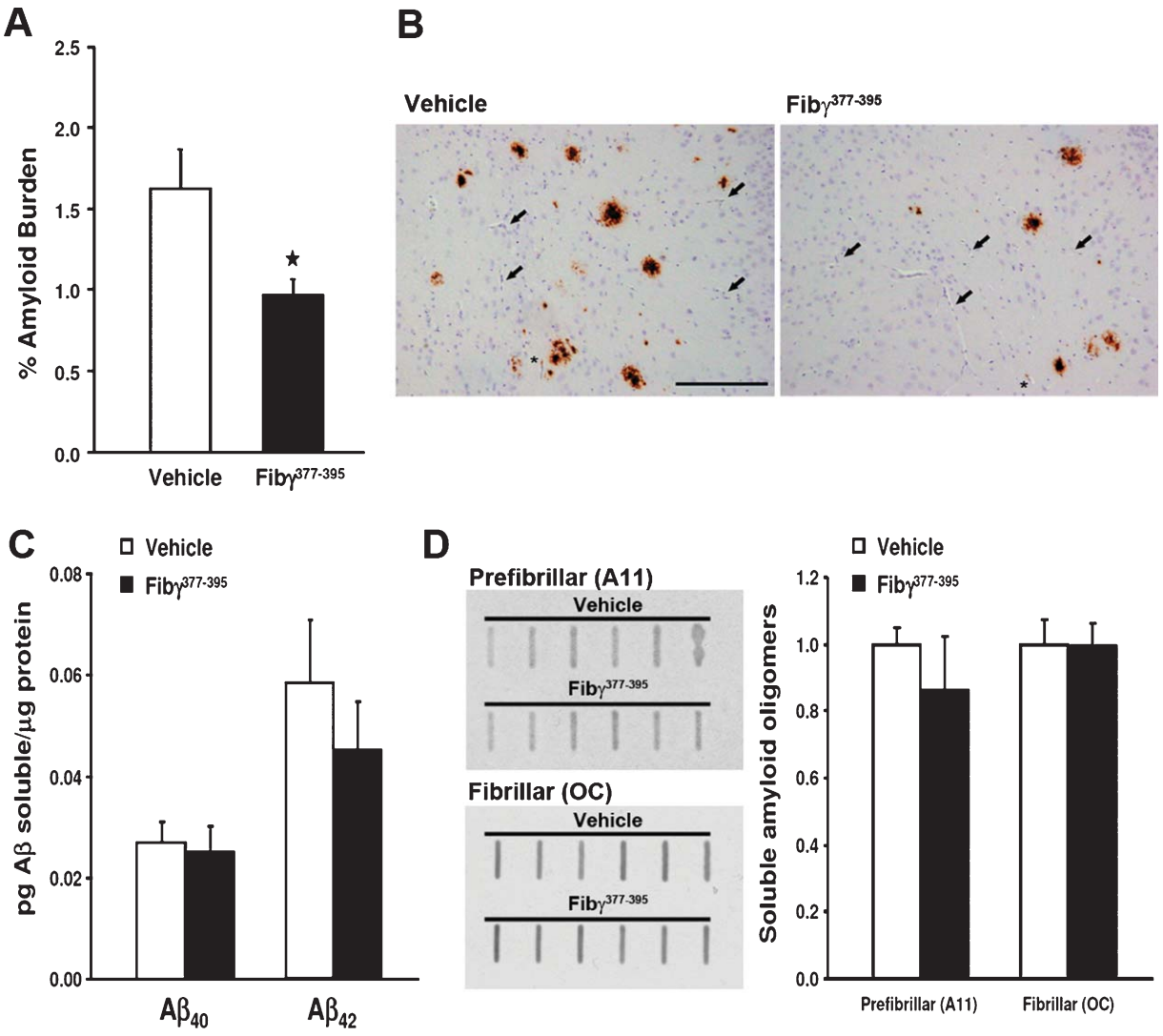

Fig. 2. A) Total Aβ burden in AβPP/PS1 mice is significantly reduced in cortex of Fibγ$^{377-395}$ peptide-treated AβPP/PS1 mice. B) Representative images of the Aβ specific immunoreactivity in cortical sections of AβPP/PS1 mice treated with vehicle (left) or Fibγ$^{377-395}$ (right). Asterisks: Aβ vascular deposition. Arrows: vessels without Aβ deposits. Scale bars represent 200 μm. C) Soluble $A\beta_{40}$ (left) and $A\beta_{42}$ (right) levels in cortical homogenates from AβPP/PS1 mice are not significantly modified by the chronic treatment with Fibγ$^{377-395}$. D) Fibγ$^{377-395}$ peptide does not modify the prefibrillar (A11 antibody) or fibrillar (OC antibody) oligomer contents in the soluble fraction of AβPP/PS1 mice cortical homogenates, as revealed by dot blot. Counts are expressed as the mean values ± SEM. ★$p < 0.05$, treatment effect.

post hoc comparisons revealed a significant increase in the vascular fibrinogen deposits in AβPP/PS1 mice treated with vehicle respect to corresponding wild-type controls ($p < 0.01$). Fibγ$^{377-395}$ peptide treatment reduced the percentage of vessels positive for fibrinogen immunostaining in AβPP/PS1 mice respect to vehicle-treated animals ($p < 0.001$) (Fig. 3). In contrast, no modification of fibrinogen staining in Aβ plaques was observed in treated AβPP/PS1 mice (Fig. 4). However, it is to note that fibrinogen staining in Aβ plaques was only revealed after formic acid pretreatment of samples and when using an Alexa546 secondary antibody against rabbit (fibrinogen) and Alexa488 secondary antibody against mouse (Aβ), since the use of the combination of an Alexa488 secondary antibody against rabbit with an Alexa546 secondary antibody against mouse resulted on no specific fibrinogen staining in Aβ plaques.

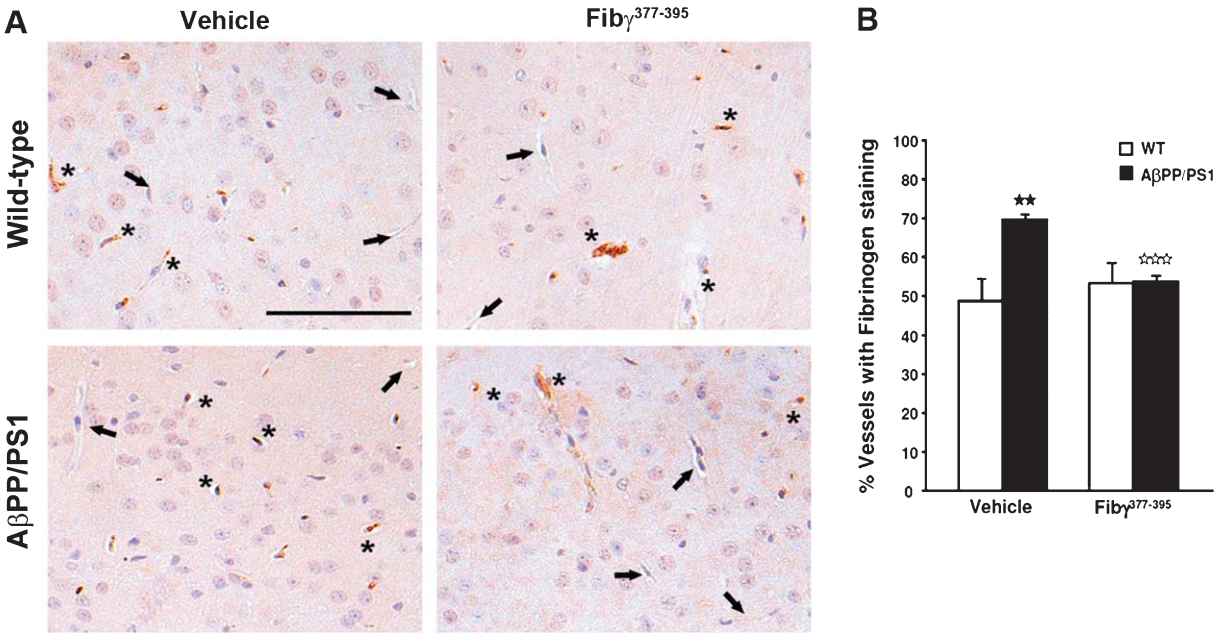

Fig. 3. Fib $\gamma^{377-395}$ peptide treatment reverses the increased percentage of vessels with fibrinogen deposition observed in AβPP/PS1 mice respect to wild-type littermates. A) Representative images of the fibrinogen vascular deposition in vehicle (left) or Fib$\gamma^{377-395}$-treated (right) mice. Scale bar represents 200 μm. Arrows: vessels without fibrinogen deposits. Asterisks: vessels with fibrinogen deposition. B) Quantification of the percentage of vessels exhibiting fibrinogen deposits. Data are expressed as the mean values ± SEM. ★★$p < 0.01$ genotype effect; ☆☆☆$p < 0.001$, compared to vehicle.

Table 2
mRNA expression levels of several cytokine-related genes involved in the inflammatory response in AβPP/PS1 mice

	Cytokine-related genes	Wild-type		AβPP/PS1	
		Vehicle	Fib$\gamma^{377-395}$	Vehicle	Fib$\gamma^{377-395}$
Anti-inflammatory cytokines	Il10ra	1.03 ± 0.11	1.00 ± 0.04	1.08 ± 0.11	1.08 ± 0.04
	Il10rb	1.01 ± 0.06	1.00 ± 0.05	1.03 ± 0.08	1.07 ± 0.03
	Tgfb1	1.02 ± 0.08	0.95 ± 0.08	1.22 ± 0.14	0.99 ± 0.08
Cell surface adhesion	Itgb2	1.06 ± 0.16	1.22 ± 0.12	1.48 ± 0.19	1.51 ± 0.10
Chemokines	Ccl3	1.03 ± 0.12	0.79 ± 0.09	5.25 ± 1.09**	3.32 ± 0.45***
	Ccl4	1.06 ± 0.17	0.96 ± 0.09	4.70 ± 1.01**	3.34 ± 0.42***
	Ccl6	1.03 ± 0.13	0.88 ± 0.10	3.04 ± 0.58**	1.99 ± 0.24**
	CxCl10	1.22 ± 0.40	1.91 ± 0.39	1.93 ± 0.34	2.73 ± 0.53
Complement system	C1ql1	1.02 ± 0.08	0.86 ± 0.09	0.74 ± 0.06*	0.89 ± 0.10
	C1qtnf7	1.04 ± 0.13	1.27 ± 0.15	1.06 ± 0.07	1.01 ± 0.10
	C3ar1	1.03 ± 0.11	1.07 ± 0.06	1.45 ± 0.18	1.30 ± 0.06*
	C4b	1.07 ± 0.16	1.10 ± 0.12	1.66 ± 0.20*	1.40 ± 0.09
Colony stimulating factor receptors	Csf1r	1.04 ± 0.13	0.94 ± 0.11	1.16 ± 0.16	1.02 ± 0.08
	Csf3r	1.06 ± 0.16	0.84 ± 0.06	1.39 ± 0.25	1.23 ± 0.10**
Fc receptors	Fcgr1	1.02 ± 0.09	1.01 ± 0.09	1.27 ± 0.13	1.12 ± 0.07
	Fcgr2b	1.03 ± 0.10	0.91 ± 0.05	1.10 ± 0.08	1.05 ± 0.04
Pro-inflammatory cytokines	Il6st	1.02 ± 0.09	1.16 ± 0.14	1.07 ± 0.11	1.07 ± 0.06
	Tnfrsf1a	1.04 ± 0.13	1.00 ± 0.18	1.18 ± 0.10	1.06 ± 0.09
Toll-like receptors	Tlr4	1.02 ± 0.09	1.23 ± 0.14	1.05 ± 0.08	1.03 ± 0.05
	Tlr7	1.03 ± 0.12	1.08 ± 0.21	1.36 ± 0.09*	1.14 ± 0.11
Others	Arpc1b	1.01 ± 0.07	0.99 ± 0.05	1.22 ± 0.06*	1.05 ± 0.04§
	Clec7a	1.08 ± 0.19	0.58 ± 0.06	6.53 ± 1.89*	4.10 ± 0.47***
	Cst7	1.06 ± 0.15	0.97 ± 0.20	33.71 ± 8.89**	20.14 ± 2.50***
	Cyba	1.03 ± 0.10	1.09 ± 0.06	1.36 ± 0.09*	1.32 ± 0.08*
	H2-Oa	1.03 ± 0.13	1.15 ± 0.06	1.60 ± 0.15*	1.57 ± 0.04***

Values are calculated with the ΔΔCt method, using the mean of three housekeeping genes (Aars, Hprt, Xpnpep1) and vehicle-treated wild-types as references. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus wild-type; §$p < 0.05$ versus Vehicle.

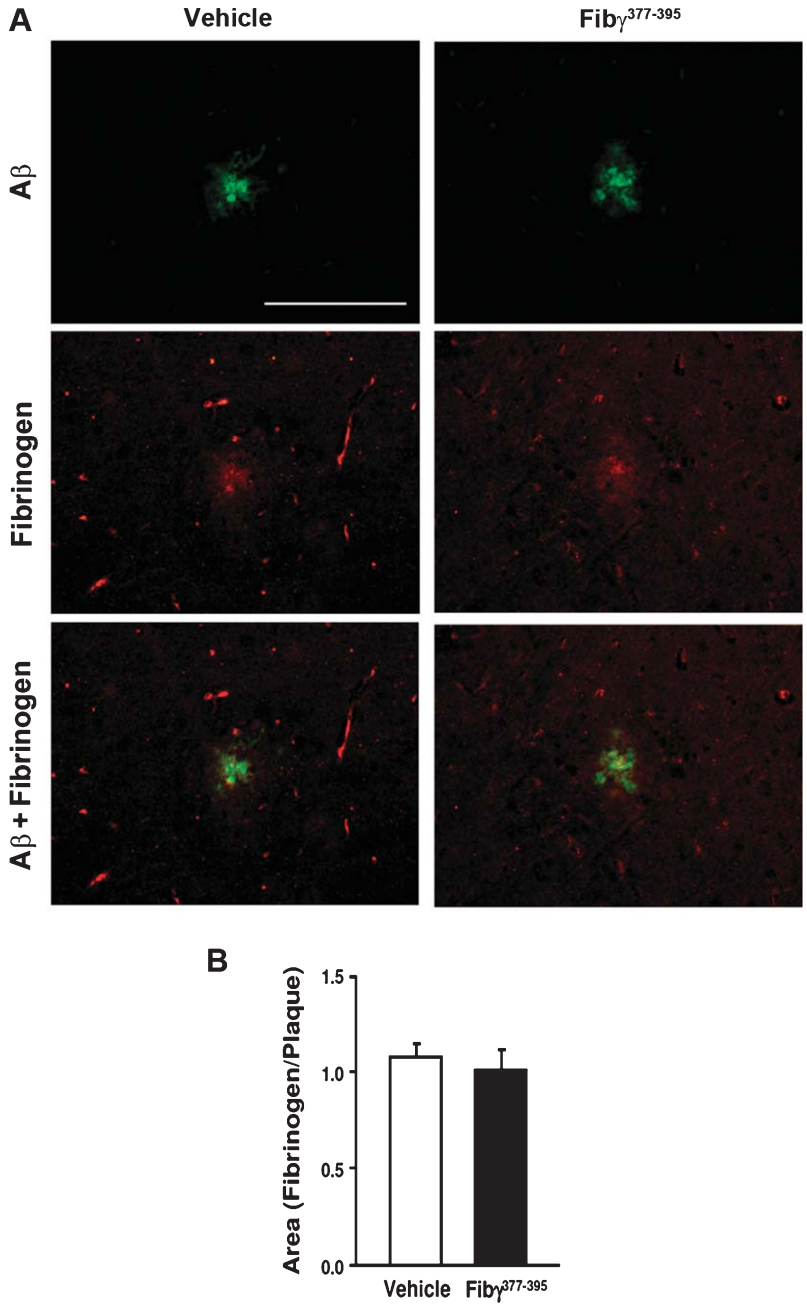

Fig. 4. Fib $\gamma^{377-395}$ peptide does not modify the fibrinogen contents in Aβ plaques of AβPP/PS1 mice. A) Representative images of the fibrinogen staining in Aβ plaques in vehicle (left) or Fib$\gamma^{377-395}$-treated (right) mice. Scale bar represents 100 μm. B) Quantification of the fibrinogen staining in Aβ plaques. Data are expressed as the mean values ± SEM.

Fib$\gamma^{377-395}$ peptide did not modify the Aβ deposition-related astroglia and microglia, or the expression of a panel of genes involved in the AβPP/PS1 inflammatory response

The number of astrocytes and microglia around Aβ plaques was not modified by Fib$\gamma^{377-395}$ peptide treatment in comparison to vehicle-treated AβPP/PS1 mice, as revealed by quantitative double-labeling immunofluorescence (Fig. 5). To assess additional molecular inflammatory changes associated with the treatment, we evaluated the expression levels of a panel of cytokine-related genes using quantitative PCR. As shown in Table 2, vehicle-treated AβPP/PS1 mice

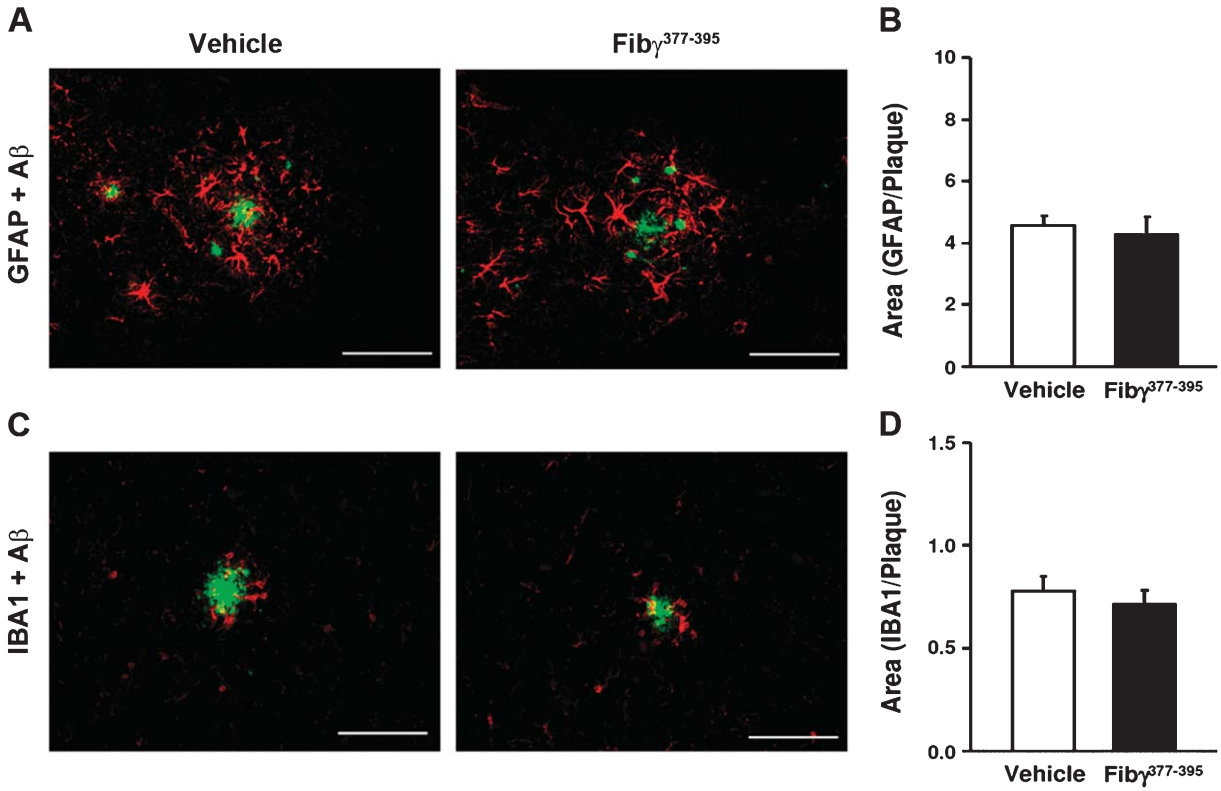

Fig. 5. Fib$\gamma^{377-395}$ peptide does not modulate glial response to Aβ deposition. A) Representative images of double GFAP (red) and Aβ (green) immunoreactivity in cortical sections of AβPP/PS1 mice chronically treated with vehicle (left) or Fib$\gamma^{377-395}$ (right). B) Quantification of the GFAP staining around the Aβ plaques reveals no significant treatment effect on the astroglial response in AβPP/PS1 mice. C) Representative images of double IBA1 (red) and Aβ (green) staining in AβPP/PS1 mice chronically treated with vehicle (left) or Fib$\gamma^{377-395}$ peptide (right). D) No significant effect on microglial response to Aβ plaques is observed on Fib$\gamma^{377-395}$ treated AβPP/PS1 mice. Scale bars represent 100 μm. Data are expressed as the mean values ± SEM.

exhibited marked modification of the expression of several neuroinflammation-related genes when compared to vehicle-treated WT animals. Treatment with Fib$\gamma^{377-395}$ did not produce significant modifications in gene expression in WT and AβPP/PS1 animals.

DISCUSSION

Fibrinogen has emerged as a putative target against vascular and Aβ pathologies in AD since it has been demonstrated that blocking its activity may reduce Aβ deposition in brain parenchyma and vessels, as well as may decrease the inflammatory processes linked to AD progression [1]. In line with these previous findings, here we provide evidence of the therapeutic properties at the cognitive level of the fibrinogen-derived peptide Fib$\gamma^{377-395}$ in an AD experimental model, the double transgenic AβPP/PS1 mice.

This peptide was known to inhibit the interaction between fibrinogen and the microglia integrin receptor Mac-1 [15] and to reduce the exacerbated inflammatory response in at least two models of degenerative diseases [9–11]. For these reasons, an anti-inflammatory effect was expected after a chronic treatment with this compound in AβPP/PS1 mice, in which an important inflammatory component is present [16]. However, no modification in the number of astrocytes and microglia, and no regulation of cytokine-related gene expression were observed in the cortex of treated animals. These results suggest that the interaction between fibrinogen and Mac-1 receptor does not play a relevant role in the microglial response in AβPP/PS1 mice and that further mechanisms of action underlie the beneficial effects observed in treated mice.

Fibrinogen is able to interact with Aβ and enhance Aβ aggregation in the vascular vessel walls and in brain parenchyma once leaked from damaged blood vessels in AD [3, 4]. We observed reduced Aβ deposition in brain parenchyma but not in blood vessel walls. However, decreased fibrinogen deposition was

found in the cerebral blood vessels in AβPP/PS1 mice treated with Fib$\gamma^{377-395}$. Previous studies have shown that this peptide is not able to efficiently inhibit the capacity of fibrinogen to bind and to aggregate Aβ peptide [3], an observation supported in the present study showing similar fibrinogen staining in Aβ plaques in treated mice. Therefore, we hypothesize that the reduction in cortical Aβ plaque burden is related to the reduction of fibrinogen leakage from damaged blood vessels in Fib$\gamma^{377-395}$-treated mice as suggested by the reduction in the percentage of blood vessels with fibrinogen deposits in Fib$\gamma^{377-395}$-treated AβPP/PS1 mice. Moreover, a direct role of the Fib$\gamma^{377-395}$ peptide on Aβ production can be probably ruled out since no effect was observed either on total soluble Aβ fraction or in prefibrillar and fibrillar oligomer contents in AβPP/PS1 mice. These observations give an advantage to Fib$\gamma^{377-395}$ over other specific Aβ-fibrinogen inhibitors recently reported to reduce vascular but not parenchymal Aβ deposition [17]. Together, the present findings point to Fib$\gamma^{377-395}$ as a potential therapeutic compound in AD based on its beneficial effect on cognition together with its capacity to retain full coagulation function [9] thus proving low undesirable side-effect profile.

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Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/14-2928r1).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-142928.

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