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ORIGINAL RESEARCH ARTICLE

β-sheet breaker peptide prevents Aβ-induced spatial memory impairments with partial reduction of amyloid deposits

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Current evidence supports the notion that β-amyloid deposits or Aβ intermediates may be responsible for the pathogenesis in Alzheimer's disease (AD) patients. In the present work, we have assessed the neuroprotective effect of the chronic intraperitoneal administration of a five-amino-acid β-sheet breaker peptide (iAβ5p) on the rat behavioral deficit induced by the intrahippocampal Aβ-fibrils injection. At 1 month after the injection, animals showed a partial reduction of the amyloid deposits formed and a decreased astrocytic response around the injection site. More importantly, we report that following the iAβ5p treatment, hippocampal-dependent spatial learning paradigms, including the standard Morris water maze and a working memory analysis, showed a significant prevention from impairments induced by Aβ deposits in the dorsal hippocampus. Thus, it is possible that a noninvasive treatment such as the one presented here with β-sheet breaker peptides may be used as a potential therapy for AD patients.
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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the deposition of Aβ amyloid in many brain regions, particularly the cerebral cortex and the hippocampus, which correlates with a progressive cognitive decline in AD patients. Growing evidence supports the notion that amyloid deposits or β-sheet-rich Aβ intermediates, including Aβ oligomers, may be responsible for the brain alterations observed in AD.[1,2] As Aβ fibrils are toxic to neurons,[3] the main targets for therapeutic intervention of the Aβ cascade include the inhibition of Aβ production, the inhibition of Aβ aggregation and fibril formation, in addition to the inhibition of the consequent inflammatory responses caused by the Aβ deposition. Several strategies, such as Aβ immunization, have been developed to prevent either amyloid fibril formation or amyloid plaques deposition. This strategy reduces the levels of Aβ, prevents and clears amyloid plaques and improves cognitive behavior in mouse models.[4–8] However, the clinic study was halted because the human vaccination induced a severe brain inflammatory response,[9,10] which can be related to cerebral hemorrhages observed after passive anti-Aβ immunotherapy in APP23 transgenic mice.[11] In addition, several molecules have been used either to inhibit polymerization of Aβ peptides or to disaggregate Aβ fibrils, including laminin[12,13] melatonin,[14] nordihydroguaiaretic acid,[15] polyphenols,[16] site-directed monoclonal antibodies,[17] α_1-antichymotrypsin,[18] fullerene,[19] *Ginkgo biloba* extract,[20] type IV collagen,[21] short Aβ congeners[22] and β-sheet breaker peptides.[23–25] β-sheet breaker peptides have both a similar sequence to the middle region of Aβ peptide and degree of hydrophobicity, but have a very low propensity to adopt a β-sheet conformation, which is responsible for the aggregation properties and the consequent neurotoxicity. Thus, these peptides have the ability to interact specifically with Aβ and block its β-sheet conformation[26] (see Figure 1), to disassemble preformed fibrils *in vitro*, to prevent neuronal death induced by fibrils in cell culture, to reduce amyloid β-protein deposition *in vivo* and to block the formation of amyloid fibrils in a rat brain model of amyloidosis.[24] These observations support the notion that these peptides may be useful in inhibiting any form of Aβ neurotoxicity and deposition *in vivo*.[27] The 5-residue β-sheet breaker peptide, iAβ5p, has been modified (acetyl-LPFFD-amide) to protect it against proteolytic degradation and to increase its blood–brain barrier

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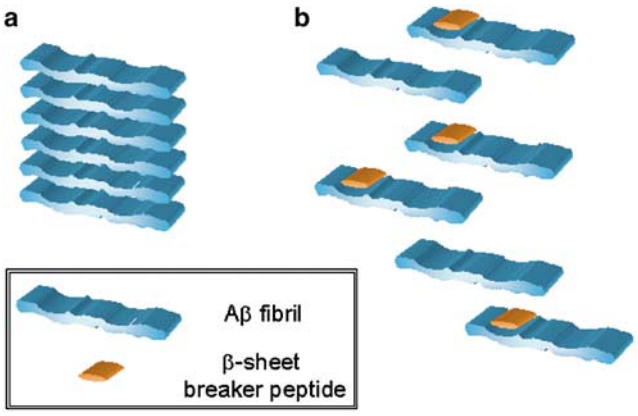

Figure 1 β-sheet breaker peptide is able to disaggregate preformed amyloid fibrils. (a) The generation of amyloid deposits occurs probably through the interaction among several amyloid fibrils. (b) In contrast, short peptides with sequence homology to Aβ (such as β-sheet breaker peptides) are able to affect this process, inducing the disaggregation of the fibrils and decreasing the formation of amyloid deposits.

permeability,[28] thus being able to reduce amyloid load and cerebral damage in a transgenic mice model of AD.[29] These modifications allow us to administer the iAβ5p peptide intraperitoneally (i.p.). However, the effects of β-sheet breaker peptides on cognitive performance and behavioral impairment induced by Aβ have not been studied, even though these peptides may become effective cognitive protectors for AD patients.

To investigate whether β-sheet breaker peptides would be able to both reduce the Aβ-amyloid deposition and improve spatial learning acquisition, we used a rat model consisting of an intrahippocampal stereotaxic bilateral injection of preformed Aβ fibrils. The injections were administered into the hippocampus to induce the formation of Aβ-amyloid deposits. Injected animals were treated intraperitoneally with the iAβ5p peptide and were subsequently analyzed in their spatial learning acquisition, using the standard Morris water maze protocol[30,31] and the working memory test.[32] After the behavioral analysis, histological analysis of the hippocampal region was performed, evaluating the amyloid deposition and detecting reactive astrocytes around the injection site. Our results show that i.p. treatment with the iAβ5p peptide partially decreases the amyloid deposition induced by Aβ and decreased the astroglial response. Both events can be related to a significant improvement of the spatial learning acquisition. The results suggest that β-sheet breaker peptides could be used as a potential therapy for AD patients.

Materials and methods

Materials

Aβ peptide corresponding to residues 1–42 of the human wild-type sequence ($A\beta_{1-42}$) was obtained from Bachem (Torrance, CA, USA). Anti-glial fibrillary acidic protein (GFAP) polyclonal antibody was obtained from DAKO (DAKO, Carpinteria, CA, USA), and anti-Aβ peptide polyclonal antibody was obtained from Sigma (Sigma Chemical Co., St Louis, MO, USA). β-sheet breaker peptide (iAβ5p) was synthesized by Neosystem (Strasbourg, France).

Injection and treatment protocol

Male Sprague–Dawley rats (280–320 g; 3 months old; 9 animals by treatment group) were anesthetized with Equitesin (2.5 ml/kg i.p.) and injected stereotaxically into the dorsal hippocampus (−3.5 mm AP ± 2.0 mm ML and −2.7 mm DV, according to Bregma using a Rat Brain Atlas)[33] with a 10 μl Hamilton syringe with 27 G stainless steel. The animals were injected bilaterally (at rate 0.5 μl/min) with 3 μl (each hippocampus) of 40 μg $A\beta_{1-42}$ fibrils formed as described previously[31] or with artificial cerebrospinal fluid (aCSF: 130 mM NaCl, 2.6 mM KCl, 4.3 mM $MgCl_2$ and 1.8 mM $CaCl_2$) as control. Briefly, fibrils were formed in PBS pH 7.4 and DMSO. After stirring for the Aβ-peptide polymerization, Aβ fibrils were precipitated by centrifugation and the pellet was resuspended in aCSF for the intracerebral (i.c.) injection. At one day after the injection procedure, animals were treated i.p. with either iAβ5p (4.5 mg/rat/injection) or saline during 1 month (3 injections per week).

Behavioral tests

After treatment, all animals were trained in a circular pool (1.6 m diameter and 75 cm deep, painted black) and the water (50 cm deep; not made opaque) was maintained at 19–21°C. Data were gathered with a video-tracking system for water maze (HVS Imagem, Hampton, UK). First, a standard water maze protocol (platform was not moved) was carried out for 2 weeks and the performance of the different groups was recorded for analysis. Briefly, rats were trained with two trials per day, for 5 consecutive days, followed by 2 days off, and then trained for 5 additional days. Rats that failed to locate the platform within the time limit were ascribed an escape latency of 90 s and were placed on the platform by hand for 5 s and then returned to its cage. Upon completion of all two trials, the rats were removed from the maze, dried and returned to their cage. For the spatial acuity parameter, the pool was subdivided by imaginary lines into four equal quadrants. These lines, in turn, intersected the edge of the pool at the arbitrary cardinal start locations named north, south, east and west. Furthermore, the pool was divided into three equidistant concentric annuli or zones. Platform was located at quadrant 4 and zone B. The percentage of permanence in specific regions of the pool was obtained from the software (HVS Imagem, Hampton, UK). Then, the product of the percentage of permanence in quadrant 4 by the percentage of permanence in zone B was calculated. Following the standard training, animals were trained with the working memory test (repeated acquisition). This test was conducted for 4 consecutive days and consisted of

five trials (one session) per day. The working memory test was procedurally similar to standard water maze training, except that the platform location was changed for each session. The rat was allowed to swim to the platform in each location and to remain there for 10 s. The rat was then placed in a home cage for an intertrial interval of 15 s. The platform remained in the same location throughout the remaining four trials of the day. Spatial working memory was regarded as the mean escape latency of the second to fifth trials. The ability of working memory in each rat was assessed by the mean performance for the 4 consecutive days of training.

Perfusion and fixation

Six rats per group were anesthetized with Equitesin (2.5 ml/kg i.p.) and perfused through the heart with saline (0.9% NaCl), followed by fixation with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) for 15 min. Brains were removed and postfixed in the same fixative for 24 h at room temperature, followed by 20% sucrose in PBS for 48 h at 4°C. After fixation, brains were coded to ensure unbiased processing and analysis. The brains were then cut into 40 μm coronal sections with a cryostat (Leitz, 1900), from Bregma −1.8 mm to Bregma −4.8 mm. Sections from the same brain were divided into groups for analysis by the following procedures: Thioflavine-S (Th-S) and Congo Red (CR) staining and immunohistochemical detection of GFAP and Aβ peptide.

Immunohistochemical staining

Free-floating immunohistochemical procedure was carried out. Washing and dilution of immunoreagents were performed with PBS with 0.2% Triton X-100 (PBS + T), and two PBS + T washes were performed between every antibody incubation. At least six sections *per* brain were used: these were pretreated with 0.5% H_2O_2 for 20 min to reduce endogenous peroxidase activity followed by treatment with 5% normal goat serum (DAKO, Carpinteria, CA, USA) at room temperature for 1 h to avoid nonspecific binding. Aβ peptide and GFAP detection was performed using either rabbit anti-Aβ or GFAP (1 : 500) polyclonal antibody, respectively, incubated overnight at 4°C. A horseradish peroxidase-conjugated goat anti-rabbit IgG second antibody (1 : 600) was used, incubated for 1 h at room temperature. Two PBS + T washing and one PBS washing were carried out before the developing step. The staining was developed by incubating during 15 min with 0.6% DAB followed by the addition of H_2O_2 (0.01% final concentration). After immunostaining, all sections were mounted on gelatin-coated slides, air-dried, dehydrated by serial rinses in graded ethanol solutions, cleared with xylene and coverslipped with Canada mounting balsam (Merck, Darmstadt, Germany).

Stainings

Th-S staining was carried out as described previously,[34] and CR staining using 'alkaline CR methods' was performed as described previously.[35] Briefly, Th-S staining was developed in sections mounted on gelatin-coated slices. After dehydration and rehydration in ethanol and xilol batteries, slices were incubated in distilled water for 10 min and then were immersed in the Th-S solution (0.1% Th-S in 70% ethanol) for 5 min. Then, slices were washed twice in 70% ethanol for 30 s and coverslipped with antifade mounting medium in dark. For the CR staining, brain sections mounted on gelatin-coated slices were dehydrated and rehydrated. They were then incubated in a solution containing 80% ethanol in NaCl saturated water for 20 min. After this incubation, slices were covered with the CR solution during 10 min. Then, the slides were washed in 70% ethanol for 5 s and transferred to absolute ethanol (twice) and xilol clearing (twice). Finally, slices were coverslipped with Canada Balsam mounting medium.

Image analysis

Six rats per group were used for the image analysis and at least three micrographs from each rat were analyzed for both amyloid and astrocyte measurements. The area of the amyloid deposit and the number of GFAP-positive astrocytes around the upper leaf of the dentate gyrus (DG) was calculated in ×40 pictures of coronal brain sections stained with Th-S or with the anti-GFAP antibody, using the SigmaScan Pro software. With the same software, the GFAP intensity in the astrocyte soma and the area of the astrocyte perikaryon were measured.

Statistical analysis

Results were expressed as mean ± standard error. Behavioral data analyses were conducted with SPSS statistical software evaluation version (www.spss.com). Statistical analyses were performed using ANOVA for repeated measures followed by Newman–Keuls *post hoc* test if appropriate. Student's *t*-test was carried out for the image analysis. Significance was accepted if $P<0.05$.

Results

iAβ5p treatment protects from spatial learning impairments induced by amyloid deposits

The analysis of the behavioral performance by the Morris water maze test showed that animals injected with $A\beta_{1-42}$ and treated with iAβ5p present significantly lower escape latency values than animals injected with $A\beta_{1-42}$ and treated with saline ($F_{(4,45)}=7.26$; $P=0.015$) (Figure 2a), indicating that iAβ5p treatment is able to reduce the cognitive impairment on spatial memory performance induced by $A\beta_{1-42}$ fibrils. In contrast, animals injected i.c. with aCSF and treated i.p. with saline or iAβ5p showed no significant difference in their escape latency values ($F_{(4,45)}=0.59$; $P=0.44$) (Figure 2b), suggesting that the

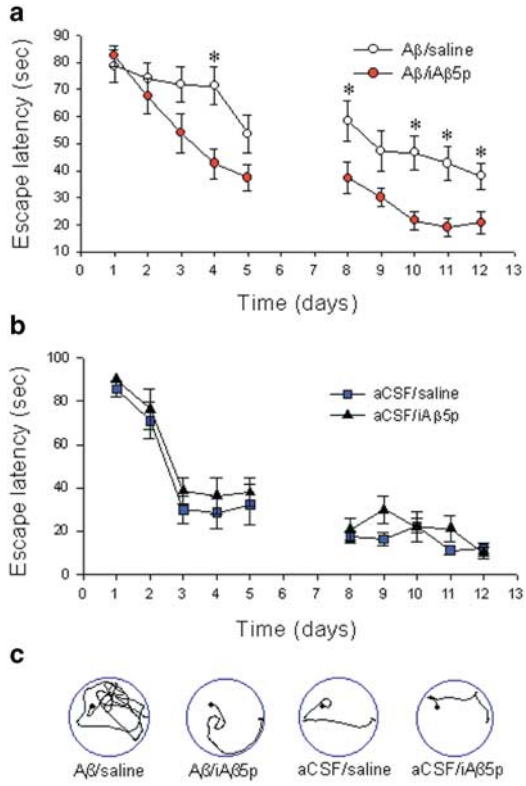

Figure 2 iAβ5p treatment decreases escape latency scores. Rats were injected bilaterally into the dorsal hippocampus with Aβ fibrils or vehicle (aCSF) and treated during 1 month with iAβ5p or saline as control ($n=9$ in all groups). (a) Behavioral performances in Morris water maze evaluated by escape latency during the 2 weeks of training. (b) Escape latency values of control animals injected with vehicle showing low escape latency values. (c) Representative swimming paths during day 8 of training. $^*P<0.05$, determined by two-way ANOVA. Each point represents the mean $\pm$ SEM of each group for a single session.

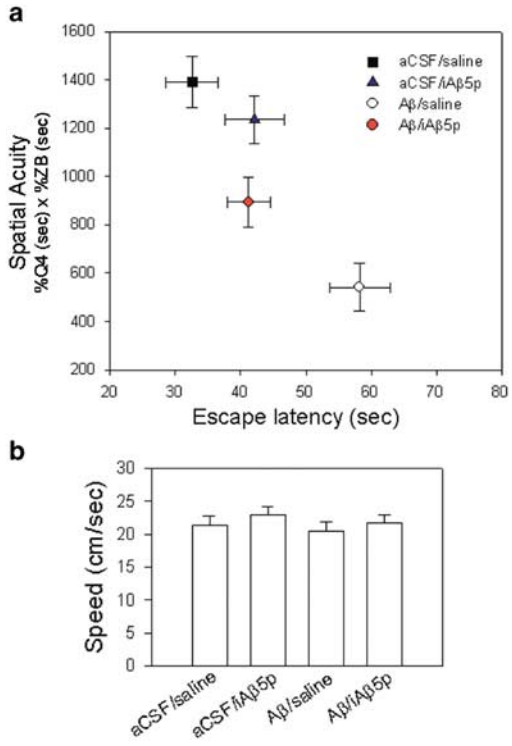

Figure 3 iAβ5p treatment improves spatial acuity and has no locomotion effects. (a) Spatial acuity versus escape latency average is shown. Spatial acuity is obtained from the product of the percentage of permanence in quadrant 4 (platform quadrant) by the percentage of permanence in zone B (platform zone), in the 2 weeks of training. (b) Swimming speed was calculated in each group, showing no significant changes in the swimming capabilities of the animals ($n=9$ in all groups). Results are expressed as mean $\pm$ SEM.

β-sheet breaker peptide *per se* neither induces spatial learning impairments nor enhances the cognitive status of the rat. Representative navigation paths at day 8 of training show the notorious impaired spatial learning acquisition of animals injected i.c. with Aβ_{1-42} and treated i.p. with saline, in comparison with those treated with iAβ5p, which display a navigation pattern similar to control animals (Figure 2c). Spatial acuity is a more sensitive parameter to measure spatial learning, representing the probability to find the rat in a specific region around the hidden platform. In Figure 3a, the relationship between spatial acuity and the escape latency average for the animals of the different groups is shown. As observed in the graph, animals injected with Aβ amyloid and treated with saline are localized in a region of the graph with high escape latency values and low spatial acuity scores, reflecting the impaired spatial memory of these animals. In contrast, animals with Aβ-amyloid deposits and treated with the β-sheet breaker peptide, iAβ5p, show low escape latency values (similar to control rats) and spatial acuity scores close to those of control animals, in agreement with the results presented in Figure 2 and supporting the protecting effect of iAβ5p on memory impairment induced by Aβ neurotoxicity. To rule out that both procedures (the i.c. injection and the i.p treatment) affect the locomotion performance, the swimming speed average (cm/s) was recorded (Figure 3b), showing no difference among the studied groups.

We also evaluated the effect of the iAβ5p peptide over repeated acquisition. For this, we used the working memory test, where the platform position was changed daily (Figure 4a) and the animals trained in each platform location trial. The results show that in the four platform locations analyzed, rats injected with Aβ_{1-42} fibrils and treated with iAβ5p showed a lower escape latency score compared to the Aβ-injected animals treated with saline ($F_{(4,06)}=7.67$; $P=0.008$). An additional parameter analyzed was the permanence in the zone B, an imaginary annulus where the platform is located. Then, this parameter was analyzed as in the classic water maze as in the working memory test (Figure 4b). As observed in the

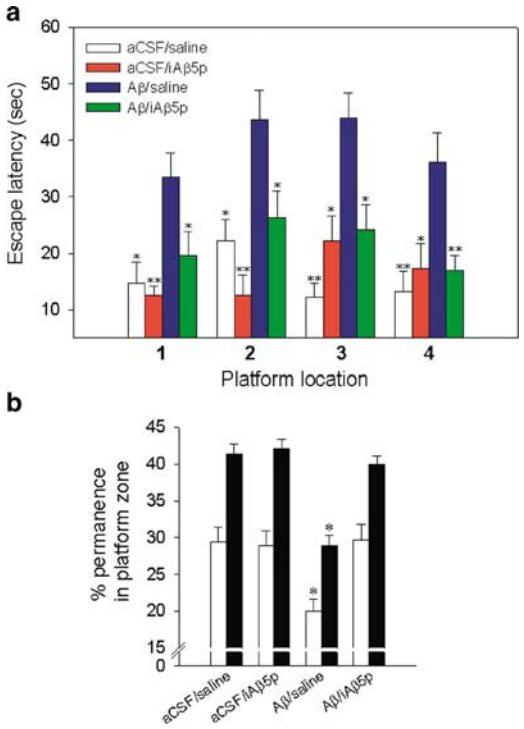

Figure 4 iAβ5p treatment improves working memory performance. The animals ($n=9$ in all groups) were trained with the working memory test and the average escape latency values were graphed. (a) As observed in the graph, animals injected with Aβ fibrils and treated with iAβ5p showed significantly lower escape latency values, in comparison to rats treated with saline. $^{*}P<0.05$; $^{**}P<0.01$ (Aβ/saline *vs* Aβ/iAβ5p), determined by two-way ANOVA analysis. No statistical differences were observed between the groups treated with aCSF (with or without iAβ5p treatment) and the rats injected with amyloid fibrils followed by iAβ5p treatment. (b) The permanence in the platform zone of the different groups trained in the classic water maze test (white columns) or in the working memory test (black columns) is shown. In both cases, the iAβ5p treatment improved the behavioral performance. $^{*}P<0.05$. Values are expressed as mean $\pm$ SEM.

graph, animals injected with Aβ_{1-42} and treated with iAβ5p showed higher permanence time in zone B, similar to control rats. In contrast, those rats injected with Aβ_{1-42} and treated with saline showed lower time of permanence ($F_{(3,9)}=5.17$; $P=0.024$, for standard water maze; $F_{(3,9)}=4.8$; $P=0.03$, for working memory test). The overall behavioral data obtained in both the standard Morris water maze procedure and working memory test demonstrate that iAβ5p is able to protect animals from memory impairments induced by intrahippocampal Aβ-amyloid deposits.

Partial reduction of induced amyloid deposits by iAβ5p treatment

With the aim to detect whether the protection in spatial memory correlated with a reduction in amyloid deposits, immunodetection of Aβ peptide and specific amyloid staining, such as Th-S and CR, were carried out. As observed in Figure 5a, c and e,

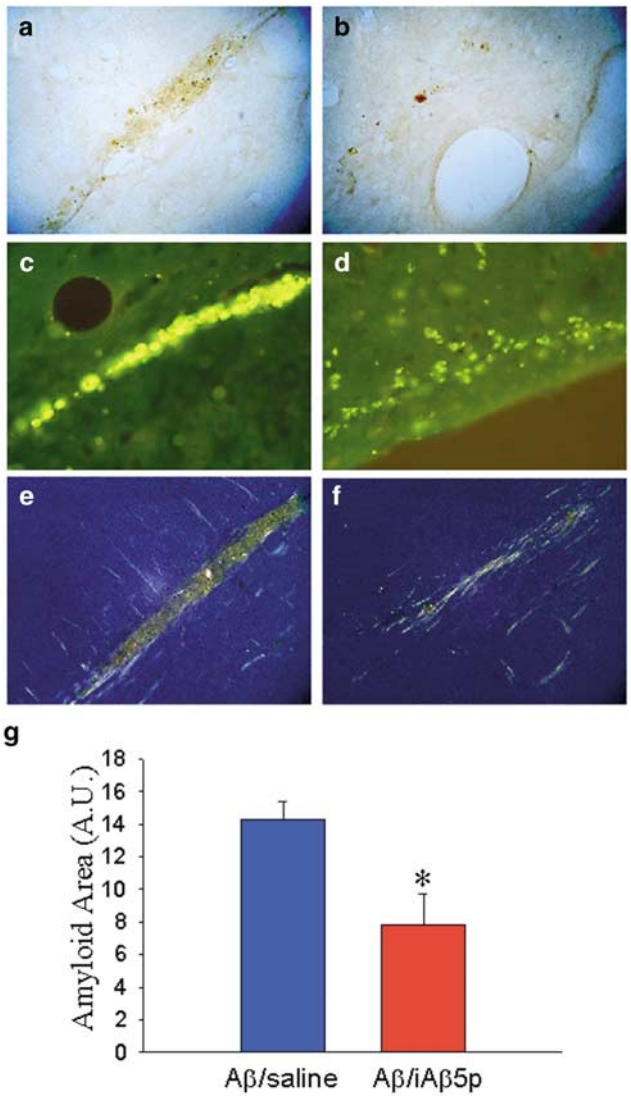

Figure 5 Partial decrease of amyloid deposition by iAβ5p treatment. Representative micrographs of amyloid deposits found in rats injected i.c. with Aβ fibrils and treated i.p. with saline (left panels) or with iAβ5p (right panels) are shown ($n=6$ in all groups). (a and b) Immunodetection of Aβ peptide. (c and d) Th-S staining. (e and f) Congo Red staining. (g) Image analysis of brain sections stained with Th-S. Area measurements were performed by SigamScan Pro software. At least three fields were analyzed from each micrograph. Statistical analysis was carried out by Student's *t*-test ($P<0.05$). Micrographs are shown at $\times 40$ magnification. Results are expressed as mean $\pm$ SEM.

saline-treated animals showed big and thick amyloid deposits in the hippocampus, as evidenced by immunoreactivity with anti-Aβ antibody, a strong fluorescence with Th-S staining and birefringence with CR staining, respectively. In contrast, amyloid deposits found in rats treated with iAβ5p are clearly reduced (Figure 5b, d and f), showing only small fragmented amyloid deposits by immunohistochemistry and staining with both Th-S and CR. The image analysis of coronal brain sections stained with Th-S

revealed a 45% reduction of amyloid burden in animals treated with iAβ5p compared to controls ($t_{(2,1)} = 2.39$; $P = 0.028$) (Figure 5g). These results support the possibility that memory deficits are associated with some form of amyloid deposits.

iAβ5p treatment does not induce astrocytic inflammatory response

In order to study a possible inflammatory reaction after treatment with the β-sheet breaker peptide, reactive astrocytes were analyzed using an anti-GFAP polyclonal antibody and a peroxidase-conjugated second antibody. Morphological brain analysis of animals injected i.c. with Aβ_{1-42} fibrils and treated with saline showed a strong GFAP staining in the upper leaf of the DG (arrow) and around this region (head arrow) (Figure 6a). Animals treated with iAβ5p also showed reactivity on the amyloid deposit (in the upper leaf of the DG; arrow), but the GFAP staining decreased around the injection site (head arrow) (Figure 6b). To clarify this issue, a deeper analysis of the reactive astrocytes was carried out; such analysis allowed us to identify hypertrophic astrocytes. Animals injected with Aβ_{1-42} fibrils and treated with either saline or iAβ5p did not show differences in the density of GFAP-positive astrocytes around the injection site (Figure 6c, d and g). However, only the reactive astrocytes found in animals treated with saline showed an increased GFAP intensity in the soma ($t_{(2,1)} = 2.198$; $P = 0.035$) and an enhanced size of the perikaryon ($t_{(2,1)} = 2.066$; $P = 0.046$) (Figure 6e and g) compared to those rats treated with the iAβ5p peptide (Figure 6f, g), indicating that the β-sheet breaker peptide blocked the activation of astrocytes to become hypertrophic glial cells in response to Aβ. These results suggest that the treatment with the iAβ5p peptide diminished the inflammatory response in the injection site, probably by decreased deposition of Aβ peptide.

Following these observations, we decided to test whether the iAβ5p peptide could trigger an astrocytic response *per se*. For this purpose, coronal brain sections of animals injected i.c. with aCSF and treated with either saline or the iAβ5p peptide were analyzed with the anti-GFAP antibody (Figure 7). Animals injected i.c. with aCSF and treated i.p. with saline (Figure 7a) or with the iAβ5p peptide (Figure 7b) showed a similar mild GFAP staining. As expected, rats treated with the iAβ5p peptide did not show differences in the density of astrocytes with respect to those treated with saline (Figure 7c, d and g), and the β-sheet breaker peptide did not induce the appearance of hypertrophic astrocytes (Figure 7e, f and g). These results indicate that the treatment with the β-sheet breaker peptide does not induce an inflammatory response in the brain *per se*.

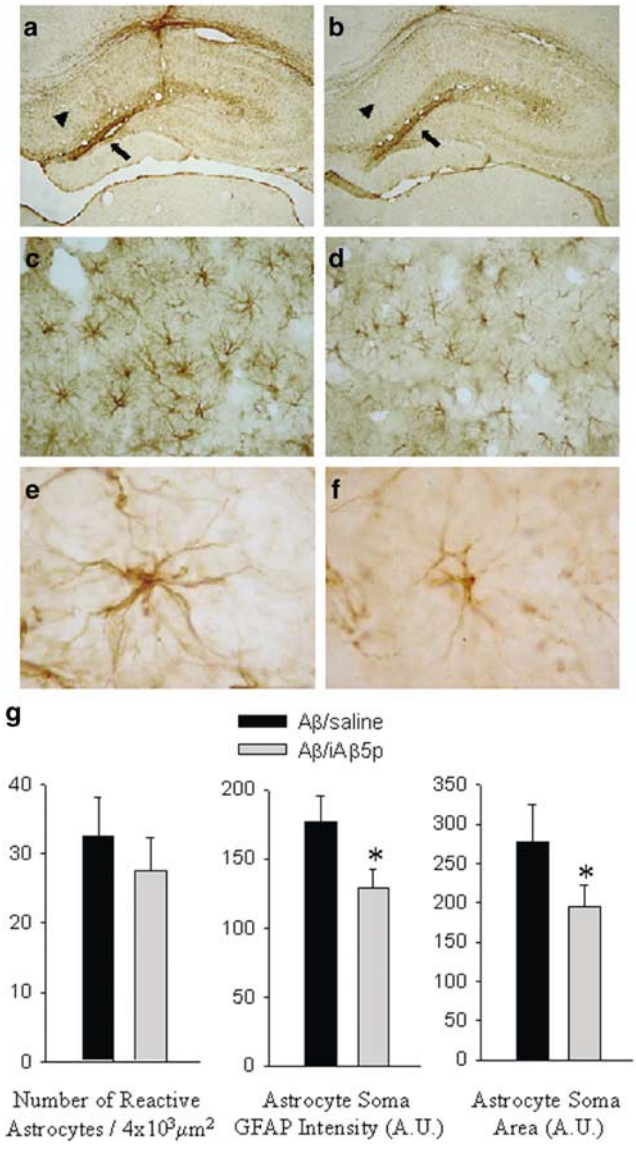

Figure 6 iAβ5p diminishes the astrocytic reactivity around the injection site. (a–f) Immunodetection of GFAP to detect reactive astrocytes in brain sections of rats injected i.c. with amyloid fibrils and treated with saline (a, c and e) or iAβ5p (b, d and f) showing that the iAβ5p peptide is able to decrease the astrocytic response induced by Aβ. (g) GFAP-positive astrocytes were analyzed on $\times 40$ pictures to determine the density of the astroglial cells, the GFAP intensity in the soma and the area of the astrocyte perikaryon, indicating that the iAβ5p peptide is able to decrease the appearance of hypertrophic astrocytes. (a, b) (c, d) and (e, f) are shown at $\times 4$, $\times 40$ and $\times 100$ magnification, respectively ($n = 6$ in all groups). Values are expressed as mean $\pm$ SEM.

Discussion

The reduction and/or disaggregation of amyloid deposits in AD brain is a promising target for treating the disease. Several strategies have been proposed to minimize or revert the negative effects of amyloid, including reduction of Aβ production, inhibition of Aβ misfolding and aggregation, enhancement of Aβ clearance and prevention of Aβ neurotoxicity.[2,27,36] Until recently, Aβ immunization was considered the

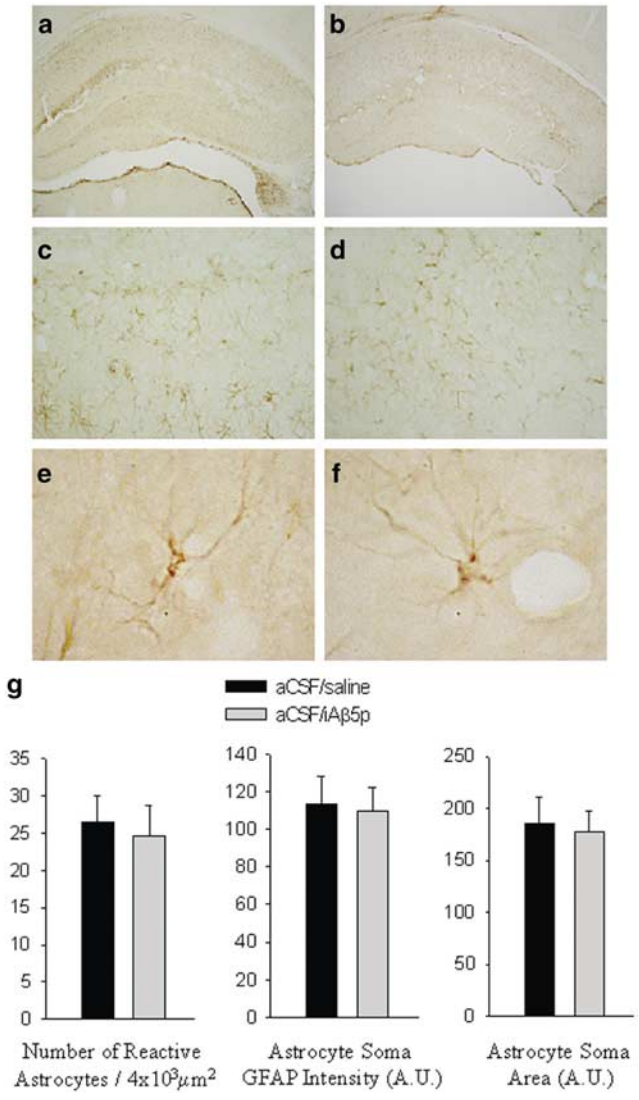

Figure 7 iAβ5p does not trigger an astrocytic response in the brain by itself. (a–f) GFAP immunoreactivity in brain sections of rats injected i.c. with aCSF and treated with saline (a, c and e) or iAβ5p (b, d and f) showing that saline, as well as iAβ5p treatment, does not induce an astrocytic response in the aCSF-injected brains. (g) GFAP-positive astrocytes were analyzed on $\times 40$ pictures to determine the density of the astrocytic cells, the intensity of the GFAP staining and the area of the astrocyte perikaryon, indicating that neither the saline nor the iAβ5p peptide treatment is able to trigger the appearance of hypertrophic signals on the astrocytes. (a, b) (c, d) and (e, f) are shown at $\times 4$, $\times 40$ and $\times 100$ magnification, respectively ($n=6$ in all groups). Values are expressed as mean $\pm$ SEM.

most promising strategy. However, a clinical study in humans has recently questioned this treatment[10] by revealing that immunized patients showed a strong brain inflammatory response,[37,38] in which new efforts have emerged for the immunization strategy, including the generation of specific antibodies against the β-amyloid peptide residues 4–10, which are able to inhibit the cytotoxicity and fibrillogenesis of Aβ.[39]

In this work, we have established that an alternative therapeutic treatment involving systemic administration of a small peptide resulted in an almost complete protection from behavioral disturbances, evaluated by working memory and standard water maze tests. These observations are correlated with a partial decrease of hippocampal amyloid deposits induced by Aβ injection. Thus, it is clear that while spatial memory impairments were prevented from reaching similar levels as those displayed by control animals, amyloid lesions were not completely removed by the treatment, suggesting that the amyloid deposits are not the only cause of the behavioral disturbances observed. This is in agreement with a recent report by Dodart and co-workers,[40] showing that immunization can reverse memory deficits without reducing the brain Aβ burden in a transgenic mouse model. This report suggests that the antibody could be increasing the peripheral clearance and/or sequestration of Aβ soluble species. The rapid reversion of memory impairment was evaluated with an object recognition task and with a holeboard memory task, tests different from the ones used in this work. From our results, it is evident that there is a reduction of the induced amyloid deposits in the rat hippocampus within just 1 month of treatment with iAβ5p. However, we cannot rule out that the spatial memory impairment observed may be induced by intermediates of Aβ fibrils, such as small oligomers of Aβ peptide, which have been demonstrated to inhibit hippocampal long-term potentiation *in vivo*.[1] It would be interesting to evaluate the effects of a longer treatment with the iAβ5p peptide over both the behavioral performance and the amyloid deposits. We do not know whether the reduction of Aβ-induced amyloid deposition by the iAβ5p peptide is a result of the Aβ-fibril disaggregation and/or of the inhibition of amyloid fibril interaction. Probably, the iAβ5p peptide is acting both ways, resulting in the reduction of the amyloid deposition in the rat hippocampus. The iAβ5p peptide can be acting either centrally over the Aβ fibrils injected by preventing the two events mentioned above or acting in peripheral clearance avoiding its accumulation. Since it is well established that wild-type rats or mice never form amyloid plaques under normal conditions, it is unlikely that an endogenous rat Aβ accumulates in the brain, because the rat Aβ has three amino-acid substitutions compared to the human Aβ sequence.[41]

The brain inflammatory responses observed in the human vaccination trial[9,10] led us to evaluate the effect of the treatment with iAβ5p at the level of the astrogliosis induced by the Aβ injection. Interestingly, iAβ5p treatment diminished the astrogliosis around the amyloid deposit and decreased the appearance of hypertrophic astrocytes; moreover, control rats treated with iAβ5p showed a very mild astrocytic reaction similar to that of control rats treated with saline, confirming a recent study with the same peptide in transgenic mice.[29] Moreover, the peptide is able to reduce the extent of interleukin-1β-positive

microglia-like cells that surround the Aβ deposits.[42] It is therefore highly possible that the treatment with iAβ5p has no significant side effects, which gives new hope for a novel therapeutic intervention for Alzheimer patients.

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