ORIGINAL ARTICLE

Sphingosin-1-phosphate Receptor 1: a Potential Target to Inhibit Neuroinflammation and Restore the Sphingosin-1-phosphate Metabolism

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ABSTRACT: ***Background:*** Recent evidence suggests that an extreme shift may occur in sphingosine metabolism in neuroinflammatory contexts. Sphingosine 1-phosphate (S1P)-metabolizing enzymes (SMEs) regulate the level of S1P. We recently found that FTY720, a S1P analogue, and SEW2871, a selective S1P receptor 1 ($S1P_1$) agonist, provide protection against neural damage and memory deficit in amyloid beta (Aβ)-injected animals. This study aimed to evaluate the effects of these two analogues on the expression of SMEs as well as their anti-inflammatory roles. ***Methods:*** Rats were treated with intracerebral lipopolysaccharide (LPS) or Aβ. Memory impairment was assessed by Morris water maze and the effects of drugs on SMEs as well as inflammatory markers, TNF- α and COX-II, were determined by immunoblotting. ***Results:*** Aβ and LPS differentially altered the expression profile of SMEs. In Aβ-injected animals, FTY720 and SEW2871 treatments exerted anti-inflammatory effects and restored the expression profile of SMEs, in parallel to our previous findings. In LPS animals however, in spite of anti-inflammatory effects of the two analogues, only FTY720 restored the levels of SMEs and prevented memory deficit. ***Conclusion****:* The observed ameliorating effects of FTY720 and SEW7821 can be partly attributed to the interruption of the vicious cycle of abnormal S1P metabolism and neuro-inflammation. The close imitation of the FTY720 effects by SW2871 in Aβ-induced neuro-inflammation may highlight the attractive role of $S1P_1$ as a potential target to restore S1P metabolism and inhibit inflammatory processes.

RÉSUMÉ: Le récepteur 1 de la sphingosine-1-phosphate : une cible potentielle pour inhiber la neuroinflammation et rétablir le métabolisme de S1P. ***Contexte:*** Selon des données récentes, un changement très important pourrait survenir dans le métabolisme de la sphingosine dans le contexte de la neuroinflammation. Les enzymes qui métabolisent la sphingosine-1-phosphate (S1P) régulent le niveau de S1P. Nous avons observé récemment que FTY720, un analogue de S1P, et SEW2871, un agoniste sélectif du récepteur 1 de S1P, protègent contre le dommage neuronal et le déficit mnésique chez des animaux à qui on a injecté de l'amyloïde bêta (Aß). Le but de cette étude était d'évaluer les effets de ces deux analogues sur l'expression d'enzymes qui métabolisent S1P (SMes ainsi que leur rôle antiinflammatoire. ***Méthode:*** Des rats ont été traités au moyen de lipopolysaccarides (LPS) ou d'Aß intracérébral. Le déficit mnésique a été évalué au moyen du labyrinthe aquatique de Morris et les effets de médicaments sur les SMEs ainsi que les marqueurs de l'inflammation, TNF-α et COX-II, ont été déterminés par technique de buvardage. ***Résultats:*** L'Aß et les LPS modifiaient de façon différente le profil d'expression des SMEs. Chez les animaux à qui l'Aß avait été injectée, le traitement par FTY720 et par SEW2871 avait des effets antiinflammatoires et restauraient le profil d'expression des SMEs, en parallèle à nos observations antérieures. Cependant, chez les animaux ayant reçu des LPS, seul FTY720 restaurait les niveaux de SMEs et prévenait le déficit mnésique, malgré les effets antiinflammatoires des deux analogues. ***Conclusion:*** Les effets bénéfiques de FTY720 et de SEW7821 observés peuvent être partiellement attribués à l'interruption du cycle infernal du métabolisme anormal de S1P et de la neuroinflammation. Les effets très semblables de FTY720 et de SEW2871 sur la neuroinflammation induite par l'Aß pourraient mettre en lumière le rôle important de S1P1 comme cible potentielle pour restaurer le métabolisme de S1P et inhiber le processus inflammatoire.

Keywords: Alzheimer's disease, FTY720, SEW2871, sphingosin-1-phosphate, sphingosine metabolism, S1P1 receptor

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Amongst interconvertible sphingolipid metabolites, ceramide and sphingosine have been shown to be involved in cell cycle arrest and apoptosis, while sphingosine-1-phosphate (S1P) contributes to cell proliferation, survival, migration and angiogenesis.[1,2] That is, the putative cellular insult following ceramides' rise as a major response to stress could be protected by S1P, at least partially.[3] Therefore S1P has been shown to provide an important molecular target in multiple sclerosis,[4] cancer and Alzheimer's disease

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(AD).[5,6] Amyloid beta(Aβ)-induced apoptosis has been empirically connected to sphingomyelin/ceramide pathways in various brain cells, including neurons,[7] oligodendrocytes,[8] astrocytes and glial cells[9] in which some underling mechanisms include calcium-dependent phospholipase A,[7] inducible nitric oxide synthase[8] and the p75 neurotrophin receptor.[10]

Whereas it has been documented sphingosine content declines[11,12] or increases[13,14] in AD, S1P expression has been reported to decrease in AD brains.[11] S1P cerebral level is strictly governed by sphingosine kinases (SphKs), producing it through sphingosines phosphorylation and also by S1P phosphatases (SPPases) or S1P lyases (SPLs) turning S1P to sphingosine, hexadecenal or ethanolamine phosphate.

Notably, little has been investigated about coincident changes in S1P synthetizing/degrading enzymes which may partly underlie the pathological shift in cerebral sphingosines in AD brains. Besides the suggestions about S1P's role in Aβ -nduced neural injury, evidence also suggests that S1P levels may influence innate immune responses[15,16] which are the critical component in AD associative neuroinflammation. Inflammatory responses have been shown to induce S1P-metabolizing enzymes (SMEs)[17] which may affect immune responses, indicating the immunomodulatory role of S1P.[18] In line with this, S1P receptor-5 activation by S1P is reported to result in transcription factor NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) repression and to maintain the immunoquiescent state of brain endothelial cells.[19]

The S1P analogue Fingolimod (FTY720; FTY), an immunosuppressive drug approved for the treatment of relapsing-remitting multiple sclerosis (MS), binds to different S1P receptors (S1PRs). However, evidence also suggests FTY blocking action on neuroinflammation depends on $S1P_1$ activation of astrocytes.[20] Basically, the benefits of FTY in MS therapy are ascribed to reducing the egress of T lymphocytes from secondary lymphoid organs through $S1P_1$ modulation. Given that neuroinflammation is a common factor found in AD and MS, $S1P_1$ is expected to be involved in FTY, ameliorating effects in AD. This may be supported by a neural viability study, indicating FTY and SEW2871 (SEW) may exert neuroprotective effects, as demonstrated in a classical *in-vitro* model of excitotoxic neuronal death.[21]

Our previous experiments performed to evaluate the effects of FTY in comparison with SEW, the selective S1P receptor 1 ligand, demonstrated significant protection against memory deficit and neural apoptosis induced by Aβ.[22,23] In this study, the efficacy of two S1P analogues were investigated to determine their ability to alleviate neuroinflammation as well as SMEs alterations in the context of memory impairment. Lipopolysaccharide (LPS) induced memory impairment was included in this survey to detect any net interaction with SMEs and the differential impact of the S1P analogues. To this end, corresponding tumor necrosis factor alpha (TNF-α) and cyclooxygenase (COX)-II alterations were assessed separately in animal models of memory deficit as induced by LPS or Aβ. Concomitantly the expression levels of SphK1, SphK2, SPL and SPPase were analyzed to estimate the simultaneous alterations in S1P metabolism and probable correlations with neuroinflammation and memory deficit.

Material and Methods

Drugs and animals

Wistar albino male rats weighing 250-300 g were housed in cages (four to five per cage) and were given food and water *ad libitum*. All animal manipulations were carried out according to the Ethical Committee for the use and care of laboratory animals of Shahid Beheshti University of Medical Sciences in compliance with the standards of the European Communities Council directive (86/609/EEC).

Lipopolysaccharide (Escherischia coli 055:B5) and Aβ 1–42 (Aβ) peptide (both from Sigma-Aldrich, USA) were dissolved in sterile 0.1 M phosphate-buffered saline (PBS); 0.1 M) at the concentrations of 5 μg/μl and 1 μg/μl, respectively. The peptide solution was then placed at 37°C for one week to obtain the aggregation. FTY720, a kind gift from Pajoohesh Darou Arya Company (Iran) and SEW2871 (purchased from Cayman Chemical) were dissolved in dimethyl sulfoxide (DMSO) 5% to the final concentration of 1 mg/ml each. All the chemicals aliquots were stored at -20 C until requirement is met.

Stereotaxic surgery and drug administration

Anesthetized rats (intraperitoneal (i.p); chloral hydrate; 400 mg/kg) were placed in a stereotaxic instrument (Stoelting, USA). A mid sagittal skin incision was made to expose the skull and using a five μl syringe (Hamilton, Reno, Nevada) microinjections of two μl Aβ or three μl LPS were performed. Injection of oligomerized soluble Aβ was carried out bilaterally in the cornu-ammonis 1 (CA1) area of the right and left hippocampus according to the following coordinates: 3.84 mm posterior to the bregma, 2.2 mm lateral to the mid sagittal line, and 2.5 mm ventral from the skull surface while LPS was injected into the right ventricle (intracerebroventricular, i.c.v.) at the coordinate of 1.0 mm anterior to the bregma, 1.5 mm lateral to the mid sagittal line and 3.6 mm ventral from the skull surface.[24] Microinjections were made in the rate of one μl per 60 s, and the needles were left in place for an additional 120 s post-infusion period to allow the appropriate diffusion of the drug from the injection site. From the day after stereotaxic surgery, as demonstrated in Figure 1 A and B, LPS and Aβ animals were assigned to FTY and SEW treatment. Lipopolysaccharide animals received i.p. injections of FTY (0.5 mg/kg/day) or SEW (0.5 mg/kg/day) for nine days. Lipopolysaccharide -injected rats were then subjected to water maze training during the days six to nine and probe test on day 10, followed by euthanization and brain sample collection. Amyloid β-injected animals were treated with i.p. injections of FTY (1 mg/kg/day) or SEW (0.5 mg/kg/day) for 14 days, as demonstrated in Figure 1 B, and were sacrificed on day 15. The corresponding control groups underwent stereotaxic surgery and received i.c.v. injections of PBS 0.1 M (corresponding to Aβ or LPS vehicle) and i.p. injections of DMSO 0.5 ml/kg or 1 ml/kg in LPS or Aβ animals respectively, as corresponding vehicles of FTY or SEW.

Evaluating learning and memory ability in animals received i.c.v. LPS

The Morris water maze (MWM) was used for determination of spatial learning and memory in this study. Briefly, a transparent Plexiglas platform (10 cm in diameter) was submerged two cm below the water surface in the center of one of the four quadrants of the water maze which was consisted of a dark circular pool (140 cm in diameter and 55 cm in height) filled to a depth of 30 cm with milk-clouded water (20 ± 1°C). The animal movement in the tank were recorded with a video tracking system (Panasonic Inc.,

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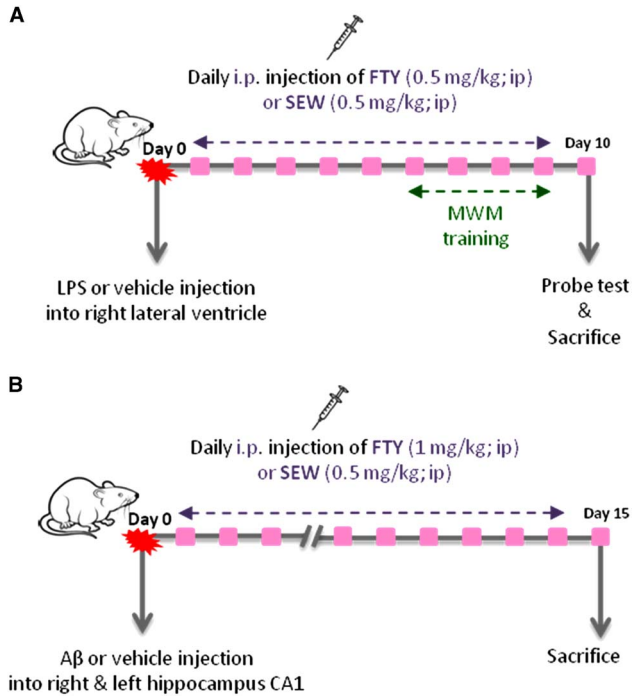

Fig. 1: *Schematic representation of experimental timelines.*

Japan) placed appropriately above the maze apparatus and analyzed by EthoVision XT 7.0 (Noldus Information Technology, the Netherlands).

The water maze training and testing were conducted on the experimental groups, each consisting of 10-12 rats, on days 6-10 after LPS or its vehicle injection and performed between 9:00 am and 12:00 am. On day 5, 24 h prior to the start of MWM training, rats were habituated to the pool by allowing them to perform a 120 s swim without the platform. During the four day training paradigm, a platform was placed one to two cm beneath the water surface and, in each trial, the rats were given 90 s to find it and a further 20 s to remain on it. Those that failed to find the platform were gently guided and placed on the platform for 20 s. The escape latency were recorded in each trial and used as measure of spatial learning. A single probe trial was conducted the day after the final training session. The escape platform was removed, and the rats were allowed to swim for 60 s in the maze. Time spent in the target quadrant were recorded and used as measures of spatial memory.

Immunoblot analysis of inflammatory markers and S1P synthetizing/degrading enzymes

Following transcardial PBS perfusion, brains were harvested from at least four rats in each experimental group and hippocampi were dissected and lysed in a Tritron containing buffer (Tris–HCl, 50 mM; NaCl, 150 mM; TritonX-100, 0.1%; sodium deoxycholate, 0.25%; sodium dodecyl sulfate (SDS), 0.1%; EDTA, 1 mM) containing protease inhibitor cocktail (Roche, Nutley, NJ, USA), using a micro-homogenizing system (Micro Smash MS-100) at 4°C for 15 min. Samples were centrifuged at 13,000 × g for five minutes at 4°C, and the supernatants were collected as total protein extracts. Total protein content in samples was determined by Bradford assay. Equal amounts of proteins (100 µg) were loaded on a 12.5% SDS polyacrylamide gel and separated by gel electrophoresis. Then proteins were transferred on polyvinylidenedifluoride membranes (Millipore). Membranes were incubated with antibody against Sphingosine Kinase 1 (Biovision; at 1:500 dilution), Sphingosine Kinase 2 (Biovision; at 1:2000 dilution), Sphingosine Phosphatase 1 (Santa Cruz; at 1:200 dilution), Sphingosine 1-phosphate lyase 1 (Abcam; at 1:10,000 dilution), COX-II (Termo Scientific; at 1:10,000 dilution) or TNF-α (Cell Signalling: at 1:200 dilution) overnight at 4°C. Blots were then incubated for 75 minutes with anti-rabbit IgG horseradish peroxidase-linked antibody (Cell Signaling, Danvers, MA, USA; at 1:10,000 dilution) at room temperature. β-Actin was immunoblotted as internal control in all samples using the β-Actin antibody (Cell Signalling). ECL Advanced Western Blotting Detection Kit (GE Healthcare) was utilized to visualize the protein bands on X-ray films which were then subjected to densitometric analysis by the Image J software.

Statistical Analysis

Data from all the experiments are expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed by one-way analysis of variance (ANOVA) (using SPSS17) followed by the Tukey HSD post hoc test. A p value of 0.05 was considered statistically significant.

Results

FTY as well as SEW prevents neuroinflammation developed by LPS or Aβ

According to our western blot analysis (Figure. 2), 10 days after LPS, or 15 days after Aβ administration, both animal groups exhibited prominent TNF-α and COX-II over expression ($p < 0.001$), indicating presence of neuroinflammation. However, in animals treated with SEW or FTY following LPS or Aβ, no difference was detectable comparing to control animals. This indicates that the S1P analogues either initially prevented the inflammatory reactions or efficiently ameliorated the elicited neuro-inflammatory responses in a few days.

LPS-induced memory impairment was not affected by SEW

Morris water maze (MWM) as a standard reliable technique for testing spatial memory is a key technique in the investigation of hippocampal circuitry,[25] Animal spatial learning (acquisition phase) and memory (probe trial) were evaluated to determine if the included treatments affected inflammation-induced injury in the hippocampus. As the MWM data at the end of training session (fourth day) shows (Figure 3, A), escape latency times turned out to be significantly less than that in the start point (first day) implying spatial learning performance in all animal groups ($p < 0.001$). Lipopolysaccharide i.c.v. injection rendered animals with a poor spatial memory compared to control ones ($p < 0.001$). As revealed by comparing escape latencies among animals received repeated FTY or SEW injections or vehicle alone, FTY could attenuate LPS-induced memory deficit ($p < 0.01$); however that was not the case for SEW. The same results were obtained with the probe test, while time duration spent in target quadrant were compared amongst animals as an appropriate index for spatial memory (Figure 3, B). Lipopolysaccharide-injected rats spent significantly less time in the

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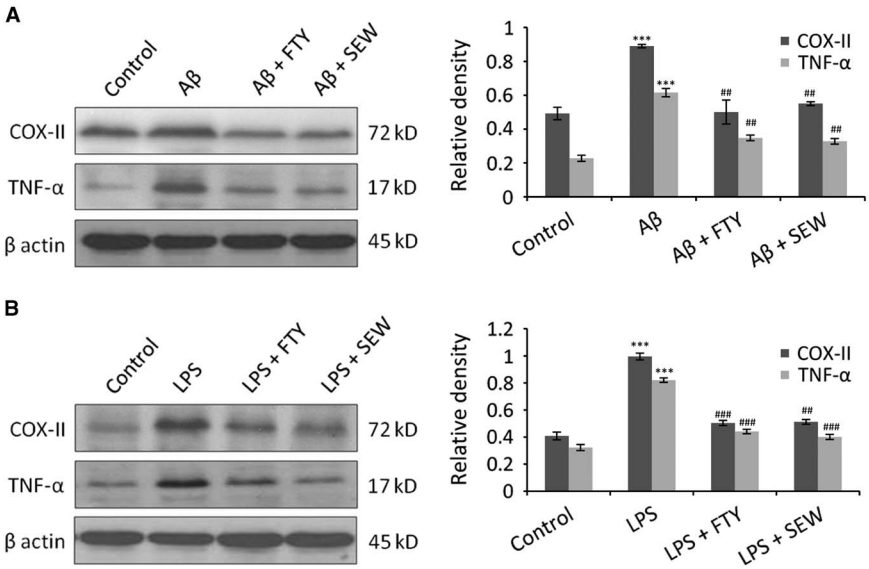

Fig. 2: *Effect of FTY or SEW on inflammatory markers induced by bilateral intra-hippocampal injection of Aβ (2 μg/2 μl) or unilateral intracerebroventricular injection of LPS (15 μg/3 μl). According to western blot assay, Aβ induced a significant enhancement in TNF-α and COX-II protein levels which were suppressed by FTY and SEW i.p. treatment (A), similar changes were observed in animals received LPS with or without FTY and SEW (B). Values are mean ± SEM (N per group: 4). ***p < 0.001 versus control; ##p < 0.01, ###p < 0.001 versus Aβ or LPS-injected animals.*

target quadrant ($p < 0.001$) in comparison with control rats. This LPS induced memory deficit was significantly restored by FTY ($p < 0.01$). The behavior observed in LPS-injected rats treated with SEW in both training and probe trials was something in between; no significant difference was observed in comparison with non-treated rats nor control animals.

Importantly, the MWM results indicate that in spite of ameliorating effects of both FTY and SEW in Aβ-induced memory deficit in our previous experiments, SEW is not efficient enough to attenuate memory impairment developed by LPS.

FTY but not SEW treatment restored all LPS induced changes in S1P metabolizing enzymes

To evaluate presumptive role of SMEs to explain ameliorating effect of FTY and SEW, we used immunoblot assay to assess the amounts of SphK1, SphK2, SPPase and SPL. Amyloid β intra-hipocampus injection in animals significantly elevated all SMEs ($p < 0.01$ and $p < 0.001$) except for SphK1, confirming less S1P available for S1PRs activation. Importantly, SPPase and SPL (S1P degrading enzymes) corresponding changes were found to be almost completely restored by both FTY and SEW treatment (Figure 4).

LPS induced memory impairment was also explored for concomitant association with S1P kinetic fluctuations. According to the blots analysis, LPS i.c.v. injection significantly enhanced SPPase ($p < 0.001$) but reduced SphK2 ($p < 0.01$) and SPL ($p < 0.001$) proteins standing for non-concordant alterations in sphingosine metabolizing enzymes. All the LPS-induced changes in the enzymes' level were almost completely restored by FTY ($p < 0.01$ and $p < 0.001$) which correlates well with its ameliorating effects on LPS-induced memory deficit. Alternatively SEW could just partially prevent LPS-induced SPL ($p < 0.05$) and SPPase ($p < 0.01$) changes of which pathological relevance to the induced memory deficit could not be evidently justified.

Discussion

Endogenous S1P molecules are transported extracellularly and gain access to their cognate receptors $S1P_1$– $S1P_5$ to act in paracrine and autocrine manner. The immunomodulatory drug FTY (fingolimod) bears structural similarity to S1P and binds to four of five S1P receptors ($S1P_1$, $S1P_3$, $S1P_4$, $S1P_5$) in comparison to SEW which is a selective $S1P_1$ ligand.[26,27]

Recently we reported a remarkable ameliorating effect for FTY and SEW, on neural injury in AD animals.[22,23] Our further works revealed FTY could also alleviate LPS-induced memory deficit as a post- or pre-treatment.[28] In the present work, we conclude that FTY or SEW could not efficiently improve memory deficit induced by Aβ or LPS, unless it is concomitant to SMEs alterations toward S1P levels equal or more than normal. In our experimental groups however, SME (SphK1, SphK2, SPL, and SPPase) alterations showed an obvious dependence on either the animal model of memory impairment or the drug (S1P analogues) we tested.

The proper balance of sphingolipids is essential for normal neuronal function. Even subtle changes in sphingolipid balance have been suggested to be intimately involved in neurodegenerative diseases including AD.[29,30] Post transcriptional levels of both SPPase and SPL have been reported to increase in AD brain as determined by the enhanced corresponding messenger RNAs,[31-33] consistently in our experiments rats' hippocampus developed enhanced levels of SPPase and SPL expression while subjected to Aβ infusion.

SPL and SPPase overexpression have been speculated to directly control cell proliferation probably through mediating

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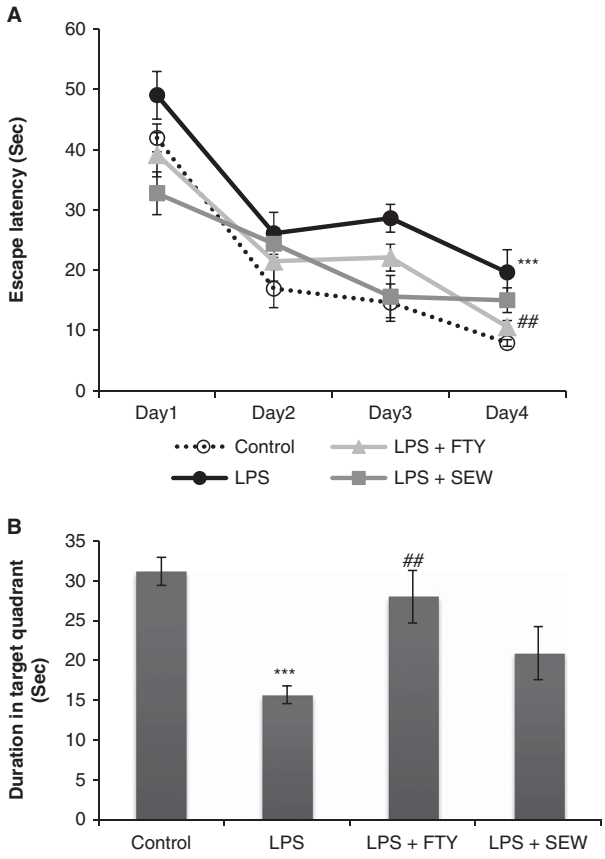

Fig. 3: *Alteration in LPS-induced memory deficit by FTY and SEW. During MWM training accomplished during four consecutive days, escape latency was used as an index for spatial learning /memory. Accordingly, FTY but not SEW treated animals showed improved memory compared to LPS animals (A). Data from MWM probe test performed following training trials indicated that LPS animals spent shorter time in the target quadrant just in comparison with FTY treated animals, indicating memory improvement by FTY (B). Values are mean ± SEM (N per group: 12). ***p < 0.001 versus control; ##p < 0.01 versus LPS-injected animals.*

apoptosis[34] in response to apoptotic stimuli like diminished intracellular S1P or enhanced ceramide levels.[35,36] In addition to such direct roles, SPL and SPPase as S1P metabolizing enzymes decreasing S1P levels may rationally disturb essential physiological content of S1P. The fact may be of more importance for SPL which irreversibly cleaves S1P to hexadecenal and ethanolamine phosphate.[37]

On the other side, SphKs including SphK1 and SphK2 creating functional pools of S1P have been identified with distinct biological functions for their different sub cellular locations. That is S1P produced by translocation of cytoplasmic SphK1 to the plasma membrane is implicated in transactivation of cell surface S1P receptors. In contrast, S1P,,made by the nuclear resident SphK2, seemingly does not trans-activate S1P receptors.[38-40,42-44] Such interpretations however may not apply to our set of experiments for the differences in subjects species (human and rat). Indeed SphK2 is the putatively predominant enzyme responsible for S1P synthesis in the mouse brain[41,42] which may also contribute to more S1PRs stimulation and the consequent protective signals. It is supported by studies, concluding anti-apoptotic properties for SphK 2[43] and other findings, suggesting SphK1 and SphK2 have at least some functional redundancy in rodents.[41,44,45,48]

Consistent with our data, Aβ plaques has been previously shown to induce SphK2 overexpression in rodents' brain.[45] Conversely, recent reports have determined significant decline in SphK1[32,33] and SphK2 in human AD brain.[32] This controversy may imply that SphK2, as the major source of S1P production, plays an active compensatory role against Aβ toxicity in rodents rather than human brain.

In accord with our behavioral examination, SEW administration restored SMEs over expression induced by Aβ which may suggest SMEs involvement in SEW ameliorating effects. In the case of FTY which did not reverse compensatory Aβ-induced SphK2, the SMEs alterations may still account for the alleviating impact since they are seemingly change towards maintaining higher S1P levels. Data we obtained here is corroborated with previous experiments indicating suppressed SPL activity following FTY treatment[46,47] which together with SphKs dependent mechanisms may affect inflammation surrounding the Aβ plaques.[48]

In spite of emerging body of investigations in AD, SMEs implication has been attended in the net context of neuroinflammation as induced by LPS. In this connection there are few suggestions about probable protective role of SphKs in LPS induced injury indicating SphK1 inhibition sensitizes raw macrophages to LPS-induced apoptosis[49-51] and worsens neuroinflammatory responses,[52] Here we showed LPS does not seem to shift SWEs towards less S1P levels but the opposite shift by FTY in the direction of enhanced S1P may provide protection against LPS-induced memory deficit.

Based on our results either FTY and SEW could suppress inflammatory markers in LPS animals more efficiently than AD ones, probably since the inflammatory status in the later, is more complicated by versatile immune reactions. Conspicuously, SEW,, in spite of FTY, was not effective in improving memory deficit. This may be accounted for by the fact that SEW not only could not reverse the SMEs changes to normal but also it appears to shift them towards less S1P content. Anti-inflammatory effects of SEW and FTY are mostly attributed to $S1P_1$ internalization and degradation, leading to rapid and dose-dependent peripheral blood lymphopenia via a $S1P_1$-mediated mechanism[53-55] while desensitizing astrocytes to external S1PRs stimuli could also partly explain such an immunosuppressive mechanism.[56]

Taken together, between the two included treatments, FTY was the optimal S1P analogue, provided efficient protection against memory impairment in AD or neuroinflammation models. While FTY impact could be at least partly explained by SMEs alterations, it should be considered FTY turning to active form upon phosphorylation by SphK2[57] may manifest diverse biological outcomes by affecting different classes of S1P receptors. Notably pre-synaptic S1P3 receptors have been shown to mediate glutamate secretion in hippocampal neurons promoting long-term potentiation and memory consolidation.[58,59]

It should be taken in to account S1P metabolism seemed to be extremely context sensitive in our experimental setting. Noting, SMEs alterations we may conclude that successful treatments could restore the SMEs changes to normal levels. On the other hand, the overall view of SMEs' concomitant changes toward more SIP production may also explain the improving effects on memory deficit. In this sense nevertheless, the weight of each

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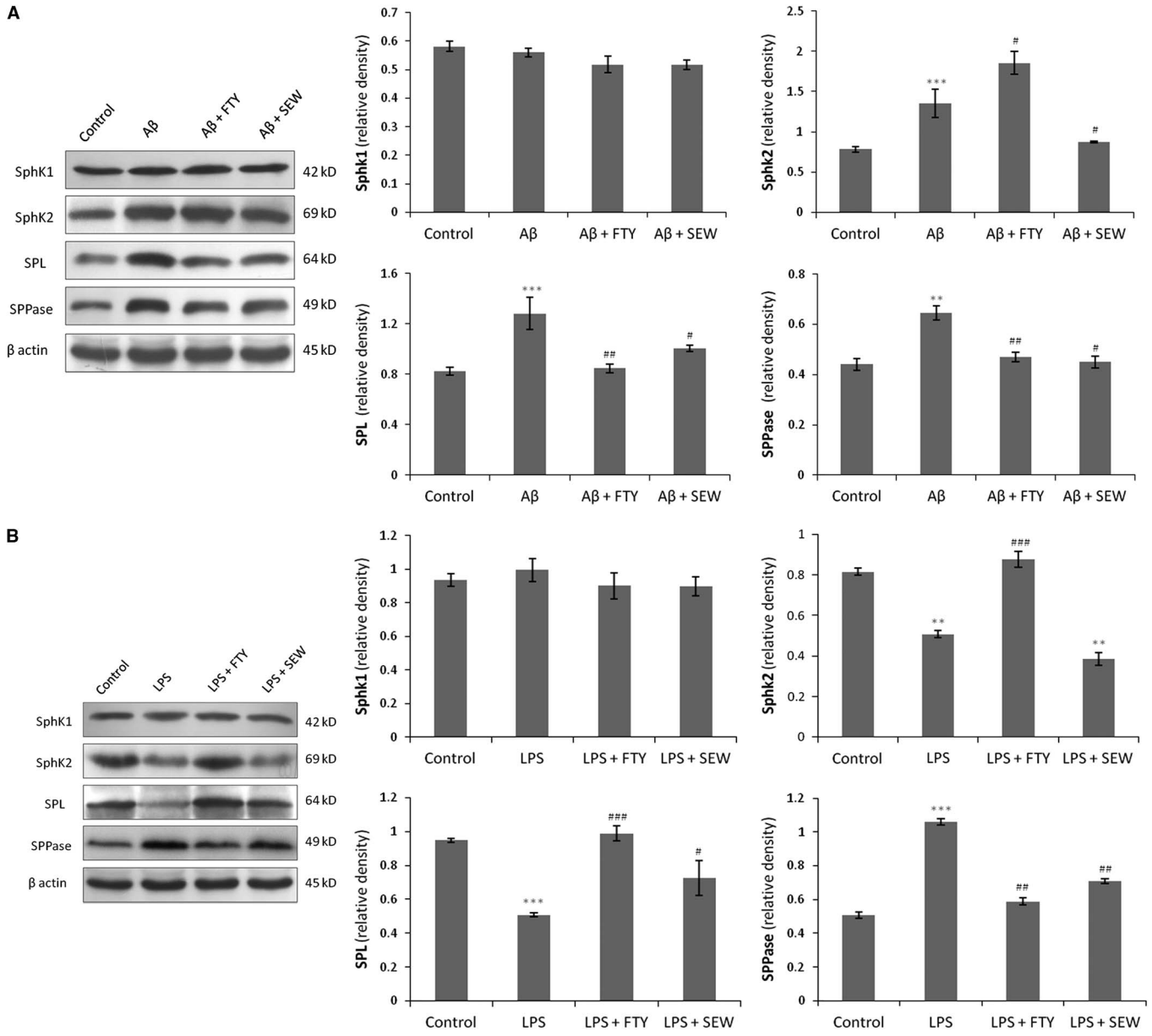

Fig. 4: *Effects of FTY or SEW on S1P metabolizing enzymes expression following bilateral intra-hippocampal injection of Aβ (2 μg/2 μl) or unilateral intracerebroventricular injection of LPS (15 μg/3 μl). Immunoblot assay of brain samples revealed a significant rise in S1P metabolizing enzymes in Aβ injected animals which were partially prevented by SEW or FTY treatment (A). LPS samples showed inconsistent changes in different S1P relevant enzymes all of which were restored by FTY but not SEW (B). Values are mean ± SEM (N per group: 4). **p < 0.01, ***p < 0.001 versus control; #p < 0.05, ##p < 0.01, ###p < 0.001 versus Aβ or LPS-injected animals*

enzyme is not clear in S1P production/degradation in animal species we utilized.

Conclusion

Fingolimod, as well as S1P metabolizing enzymes, particularly Sphks may affect Aβ production[45,60,61] which may lead to modulating oxidative stress in AD brains.[62] Accurate regio-specific evaluation has revealed SMEs correlate with AD pathology particularly in brain regions that are affected earlier in AD (i.e. hippocampus). This might highlight SMEs alterations as a diagnostic marker, in spite of evidences that have raised uncertainty about correlation between S1P and Aβ aggregations.[32,33] In the present study SMEs alterations were shown to link and explain the therapeutic efficiency of FTY and SEW to improve memory deficit. The probable involvement of other mechanisms like excitotoxicity amelioration[21] or brain-derived neurotrophic factor production[63] by FTY needs to be investigated to retain protection in AD context.

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Disclosures

None of the authors have anything to disclose.

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