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Atorvastatin and Amyloid-Beta$_{1-40}$ Promote Differential Gene Expression of Proteins Involved on Glutamatergic Transmission in the Cerebral Cortex and Hippocampus

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Abstract

Deposition of amyloid-beta (Aβ) peptides into specific encephalic structures involved in learning and memory is an important event related to Alzheimer's disease (AD) pathogenesis. As a consequence, deregulation of cellular processes such as glutamate transport, oxidative stress, neurotrophic factors levels may occur and cause synaptotoxicity and neuronal cell death. Herein, we evaluated the gene expression of proteins involved with the progression of AD. We aimed to assess an early event (after 24 h) of Aβ$_{1-40}$-induced toxicity in hippocampi and frontal cortices of mice. The effects of a pretreatment with atorvastatin, a HMG-CoA reductase inhibitor, in preventing Aβ$_{1-40}$-induced gene expression alterations were also evaluated. Atorvastatin (10 mg/kg/day, orally) was administered through 7 consecutive days before Aβ$_{1-40}$ administration. Atorvastatin treatment increases mRNA levels from NMDA receptor GluN1 subunit, PSD-95, BDNF and glutamate transporters (GLAST and GLT-1) in the cortex. In the hippocampus, atorvastatin did not alter glutamatergic genes (GluN1, PSD-95, GLT-1 and GLAST), but it reduced NOS1 and increased BDNF gene expression, what may be related to its neuroprotective action. Aβ$_{1-40}$ reduced gene expression of glutamatergic proteins and NOS1, but it increased BDNF mRNA levels, what might be interpreted as a compensatory response to Aβ$_{1-40}$ toxicity. Atorvastatin plus Aβ$_{1-40}$ treatment almost always show an opposite effect from the observed in Aβ$_{1-40}$-treated groups, reinforcing the data on the neuroprotective effect of atorvastatin against Aβ-induced toxicity even in early molecular alterations.

Keywords: Atorvastatin; Amyloid-β peptide; Glutamate; NMDA receptors; BDNF; Neuroprotection

Abbreviations

Aβ: Amyloid-Beta; AD: Alzheimer's Disease; BDNF: Brain-Derived Neurotrophic Factor; GLAST: Glutamate-Aspartate Transporter; GLT-1: Glial Glutamate Transporter; GluN1: N-Methyl-D- Aspartate (NMDA) Receptor Subunit GluN1; i.c.v.: Intracerebroventricular; NMDA: N-Methyl-D-Aspartate; NOS1: Neuronal Nitric Oxide Synthase Isoform; PSD-95: Post-Synaptic Density Protein-95 KDa

Introduction

Alzheimer's disease (AD) is a dementia characterized by progressive memory impairment leading to loss of synapses and consequent neuronal death. AD histopathology consists of two well-known hallmarks: senile plaques formed from the aggregation of amyloid-beta (Aβ) peptide and neurofibrillary tangles induced by tau hyperphosphorylation [1].

The deposition and aggregation of oligomeric species of Aβ in brain areas involved in cognitive functions initiate the cascade that results in dysfunction of many cellular processes resulting in synaptotoxicity and neuronal cell death [2,3]. Aβ species are composed of 40 to 42 amino acids and both forms have been identified in the brain of AD patients [4]. We have previously shown that an intracerebroventricular (i.c.v.) infusion of aggregates of Aβ$_{1-40}$ induce neuroinflammation, synaptotoxicity and unbalance of glutamatergic transmission and antioxidant defenses [5,6].

Additionally, it has been shown that isoforms of the nitric oxide producing enzyme, nitric oxide synthase (NOS), may display aberrant patterns of expression in AD brains, thus increasing reactive oxygen (ROS) and nitrogen species (RNS) levels and may lead to inflammation processes [7]. It is also known that excitotoxicity promoted by a dysfunction in glutamate receptors and its transporters may improve cognitive decline in patients with AD [2]. Furthermore, loss of intracellular calcium homeostasis, changes in cell signaling pathways and decreased levels of neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) are involved with the augment of this disease progression [8,9]. However,

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it is not clear whether these alterations are direct involved in triggering the degenerative process. Atorvastatin, a member of the statins family, initially pharmacologically used as a HMG-CoA reductase inhibitor to control hypercholesterolemia, has been suggested as a potential drug in the treatment of AD [10]. Although some controversial data about the effects of statins [11], studies demonstrate the pleiotropic effects of statins, as anti-inflammatory and neuroprotective in several pathological conditions of the central nervous system (CNS), as in ischemia, seizures, depressive-like disorders and Parkinson´s disease models [10,12-14]. Of particular importance, we have shown that the administration of atorvastatin (10 mg/kg, p.o.) for 7 consecutive days after i.c.v. infusion of Aβ$_{1-40}$ abolished hippocampal cell death, neuroinflammation and oxidative stress in mice hippocampus [15]. Moreover, the administration of atorvastatin for 7 days before i.c.v. infusion of Aβ$_{1-40}$ prevented cognitive deficits induced by Aβ toxicity. Additionally, the infusion of Aβ$_{1-40}$ in mice promoted increased activity of antioxidant enzymes activity in the cerebral cortex but not in the hippocampus after 16 days. These alterations may lead to changes in levels of free radicals in neuronal cells of these animals, whereas pretreatment with atorvastatin prevents this effect, suggesting that statin could regulate the production of ROS induced by Aβ [6]. These data demonstrate that treatment with atorvastatin may exert different effects in specific brain regions and in a time-dependent manner. However, studies evaluating early events involved in Aβ$_{1-40}$-induced toxicity are still scarce in the literature.

Therefore, in the present study we investigated the putative alterations in gene expression of proteins directly involved with AD progression. A putative early toxic event induced by Aβ was assessed in cerebral cortex and hippocampus after 24 h of Aβ$_{1-40}$ i.c.v. infusion in mice. Cortical and hippocampal gene expression of N-methyl-D-aspartate (NMDA) receptor subunit GluN1, its anchoring protein of post-synaptic density-95 (PSD-95), the neuronal isoform of NOS (NOS1), BDNF and the glial glutamate transporters, GLT-1 and GLAST, were measured. Moreover, we evaluated whether the pretreatment with atorvastatin is able to prevent the effects of Aβ$_{1-40}$, elucidating some potential mechanisms mediating atorvastatin-promoted neuroprotection.

Experimental Procedures

Animals

Male adult Swiss albino mice (3 months old/45 g to 55 g) were kept on a 12-h light/dark cycle (lights on at 07.00 AM) at a constant temperature of 22°C ± 1°C. They were housed in plastic cages with tap water and commercial food *ad libitum*. All procedures were carried out according to the institutional policies on animal experimental handling, designed to minimize suffering and limit the number of animals used and were approved by local Ethical Committee for Animal Research (CEUA/UFSC-PP00955). All experiments were performed during the light phase (between 9:00 and 17:00 h) to avoid circadian variations.

Drug treatment protocol

Human Aβ$_{1-40}$ (Tocris, Ellisville, MO, USA) was prepared as a stock solution at a concentration of 1 mg/ml in sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and aliquots were stored at -20°C. Aβ solutions were aggregated by incubation at 37°C for 4 days before use, as described previously [6]. Previous analysis by transmission electronic microscopy showed this Aβ$_{1-40}$ preparation contains fibrils and protofibrils, in a mixture of low molecular weight species [16]. The Aβ$_{1-40}$ peptide (400 pmol/mouse) or PBS (vehicle) was administered i.c.v. as previously described [5,13] and detailed as follows. Mice were anaesthetized with isoflurane 0.96% (0.75 CAM; Abbott Laboratórios do Brasil Ltda., RJ, Brazil) using a vaporizer system (SurgiVet Inc., WI, USA) and then gently restrained by hand for i.c.v. injections. The sterilization of the injection site was carried out using gauze embedded in 70% ethanol. Under light anesthesia (i.e. just that necessary for loss of the postural reflex), the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye and 1 mm posterior to a line drawn through the anterior base of the eyes (used as external reference). A volume of 3 µl of Aβ$_{1-40}$ or PBS solution was injected into the lateral ventricle, at the following coordinates from bregma: anteroposterior (AP)=-0.1 mm, mediolateral (ML)=1 mm, and dorsoventral (DV)=-3 mm. The accurate placement of the injection site (needle track) was confirmed at the moment of dissection of the animals for the execution of biochemical experiments. Results from mice presenting any sign of cerebral hemorrhage were excluded from the statistical analysis (overall, less than 5% of the total animals used).

To study the putative modulation of atorvastatin on the molecular changes induced by Aβ$_{1-40}$, animals were treated orally (p.o.) with atorvastatin (Lipitor Atorvastatin calcium, Pfizer) 10 mg/kg/day, once a day during 7 consecutive days (the last dose was administered 3 h before the i.c.v. administration of Aβ$_{1-40}$ or PBS) [6]. Control animals were treated with vehicle (NaCl 0.9%) orally for the same period. The treatment was done by the administration of 10 µl/g weight of the animal, both for the solutions of atorvastatin and saline. Thus, this study consists of four experimental groups: 1) Control group; 2) Atorvastatin-treated mice (Ator) group; 3) Aβ$_{1-40}$-treated mice (Aβ$_{1-40}$) group; 4) Atorvastatin plus Aβ$_{1-40}$-treated mice (Ator+Aβ$_{1-40}$). The experimental protocol of this study is summarized at Figure 1.

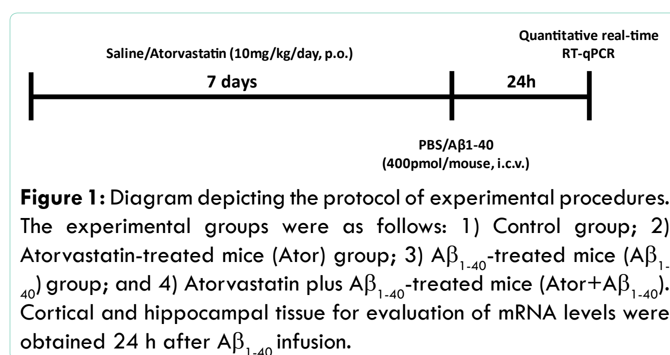

Figure 1: Diagram depicting the protocol of experimental procedures. The experimental groups were as follows: 1) Control group; 2) Atorvastatin-treated mice (Ator) group; 3) Aβ$_{1-40}$-treated mice (Aβ$_{1-40}$) group; and 4) Atorvastatin plus Aβ$_{1-40}$-treated mice (Ator+Aβ$_{1-40}$). Cortical and hippocampal tissue for evaluation of mRNA levels were obtained 24 h after Aβ$_{1-40}$ infusion.

RNA isolation and cDNA synthesis

Mice were killed by decapitation, the brain was removed from the skull and frontal cortices and hippocampi were dissected, frozen in liquid nitrogen and stored at -80°C. Samples were fragmented by adding Trizol (Invitrogen) and RNA extraction was performed following the manufacturer's instructions with minor modifications. The concentration and quality of RNA was estimated by spectrophotometric reading at 260 nm and calculated the ratios 260/280 and 260/230, respectively (NanoDrop ND -1000 Spectrophotometer). Integrity of extracted RNA was evaluated in 1.2% agarose gel. RNA samples were treated with DNase (deoxyribonuclease I, Invitrogen) to remove genomic DNA residues. Reverse transcription reaction was performed using 1 ug of total RNA. The reaction mixture consisted of: Oligo dT (12-18) (500 ng), dNTP mix (0.2 mM), 1X First-Strand Buffer, DTT (5 mM), RNaseOUT (40 U/uL), Superscript III RT (200 U/uL) (Invitrogen) and RNase free water. The reaction was performed according to the manufacturer's instructions by incubation for 1 h at 50°C followed by 15 min at 70°C. Samples were maintained at -20°C until use.

Quantitative real time RT-PCR

To evaluate the efficiency of the amplification reaction for each target gene, serial dilutions were made from a cDNA pool containing all samples. The measured concentrations were 1:10, 1:20, 1:40, 1:80, 1:160. The samples were amplified in duplicates according to manufacturer's recommendations. The reactions were prepared using standard RT-qPCR reagent (SYBR Green PCR Master Mix, Thermo Fisher Scientific) in the presence of the primer set for each gene (Table 1). cDNA of 2 uL were amplified in a final volume of 10 uL reaction. Alternatively, the sample was replaced by water (NTC) to confirm amplification specificity. The quantitation analyzes were based on 2-ΔΔCq method, with the endogenous control of GAPDH gene [17]. The stability of the control gene was verified by RefFinder online tool.

Statistical analysis

All values are expressed as means ± SD. The statistical evaluation of the results was carried out using two-way analysis of variance (ANOVA) with pretreatment and treatment as the independent variables. Following significant ANOVAs, post-hoc comparisons were performed using the Newman-Keuls test. The accepted level of significance for the tests was P<0.05. All tests were performed using the Statistica® software package (Stat Soft Inc., Tulsa, OK, USA).

Results

mRNA levels of NMDA receptor and PSD-95

NMDA receptor subunit GluN1 mRNA expression in the cerebral cortex of mice showed a significant increase in atorvastatin-treated group (Figure 2A). Infusion of Aβ$_{1-40}$ did not change the basal (control) gene expression of this protein in this cerebral structure, while the presence of both treatments (Ator+Aβ) decreased GluN1 mRNA bellow control levels.

Atorvastatin or Aβ$_{1-40}$ treatment increased the anchoring NMDA receptor protein, PSD-95 mRNA levels in the cortex, while Ator+Aβ group maintained these levels similar to control group (Figure 2B).

In the hippocampus, Aβ$_{1-40}$ promoted a decrease in GluN1 (Figure 2C) and PSD-95 (Figure 2D) mRNA levels. Pretreatment with atorvastatin prevented these reductions promoted by Aβ$_{1-40}$ infusion (Figure 2C and 2D).

mRNA levels of NOS1

No alteration in the mRNA expression of NOS1 in the group pretreated with atorvastatin was observed in the cortical region. However, mice infused with Aβ$_{1-40}$ presented a slight reduction in relation to control group in the cortical region. And, mice that received atorvastatin plus Aβ$_{1-40}$ (Ator+Aβ) presented a significant reduction in levels of NOS1 mRNA (Figure 3A). In the hippocampal region, atorvastatin, Aβ$_{1-40}$ and atorvastatin plus Aβ$_{1-40}$ groups showed a decreased mRNA expression of NOS1 in relation to control group (Figure 3B).

Target Gene	Genbank Number	Primer forward (F)/reverse (R)	Amplicon (pb)
GAPDH	NM_008084.3	(F) CATCACTGCCACCCAGAAGACTG (R) ATGCCAGTGAGCTTCCCGTTCAG	153
BDNF	NM_001048141.1	(F) CATAGACAAAAGGCACTGGAACTC (R) TAAGGGCCCGAACATACGAT	62
PSD-95	D50621.1	(F) GGACATTCAGGCGCACAAG (R) TCCCGTAGAGGTGGCTGTTG	59
NMDA	BC039157.1	(F) CACACAGGAGCGGGTAAACA (R) TCTCCCATCATTCCGTTCCA	57
GLT-1	NM_001077514.3	(F) TCTGAGCTGGACACCATTGACT (R) CGTCTTGGTCATTTCGATGTCTT	63
GLAST	AK045716.1	(F) GCTCACGGTCACTGCTGTCA (R) CGGTCGGAGGGCAAATC	55
NOS1	NM_008712.3	(F) TCAAGGGTATTTGGGTGACATTT (R) GGAATGAAAAGCAGCTACCCTATT	119

Table 1: Genes designed for RT-qPCR, Genebank accession number, sequence of the primer sets (5' → 3') employed in the amplification reaction and the expected size of each amplicon.

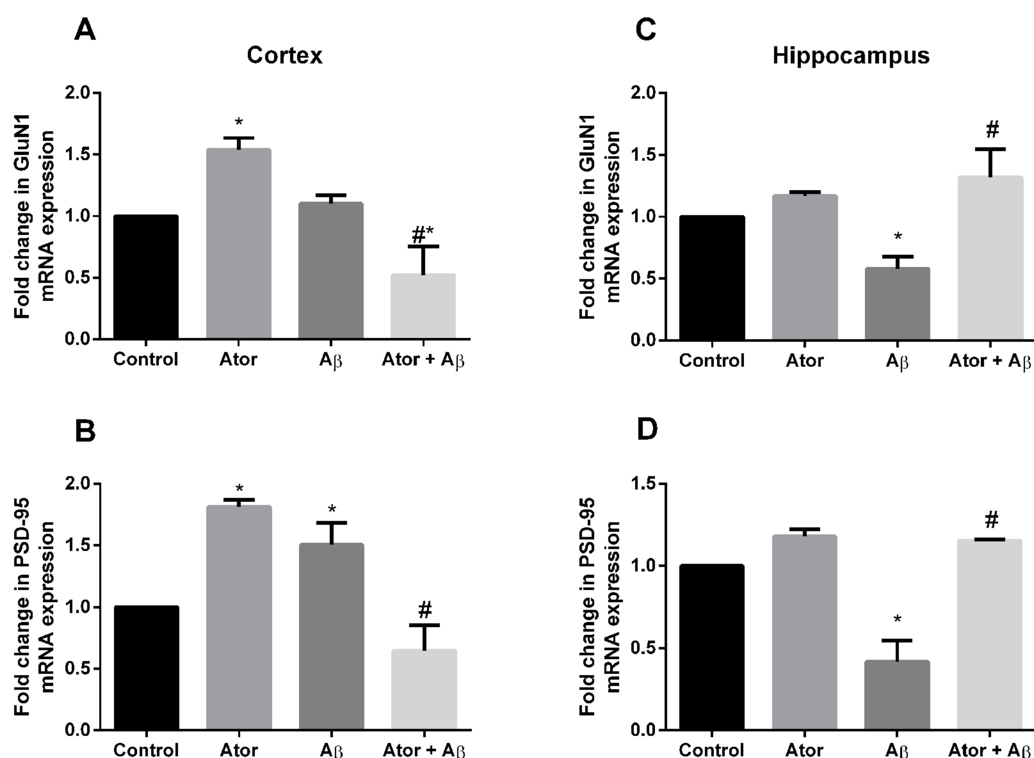

Figure 2: Evaluation of NMDA receptor subunit GluN1 and PSD-95 mRNA expression in cortex and hippocampus from mice subjected to atorvastatin pretreatment and i.c.v. infusion of A$\beta_{1-40.}$ The animals received a 7-day pretreatment with atorvastatin (10 mg/kg, p.o.) or saline and a single i.c.v. infusion of Aβ_{1-40} (400 pmol/mouse) or PBS. The tissue samples were obtained 24 h after Aβ_{1-40} infusion. Graphs represent the NMDA receptor subunit GluN1 and PSD-95 mRNA expression in the cortex (A and B) and hippocampus (C and D), respectively. Results are presented as means ± S.D.
* indicates significant difference from control group, $p<0.05$, # indicates significant difference from Aβ_{1-40} group, $p<0.05$ (Two-way ANOVA followed by Newman-Keuls post hoc test).

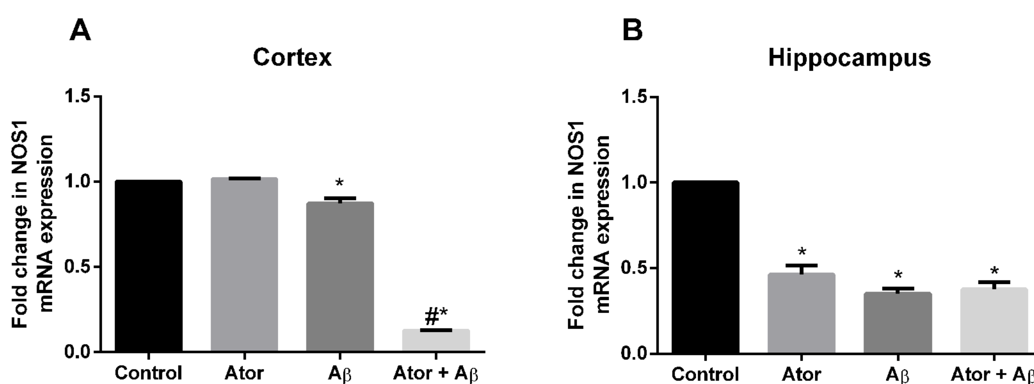

Figure 3: Evaluation of NOS1 mRNA levels in cortex and hippocampus from mice subjected to atorvastatin pretreatment and i.c.v. infusion of A$\beta_{1-40.}$ The animals received a 7-day pretreatment with atorvastatin (10 mg/kg, p.o.) or saline and a single i.c.v. infusion of Aβ_{1-40} (400 pmol/mouse) or PBS. The tissue samples were obtained 24 h after Aβ_{1-40} infusion. NOS1 mRNA expression were evaluated in cortex (A) and hippocampus (B) regions. Results are presented as means ± S.D.
* indicates significant difference from control group, $p<0.05$, # indicates significant difference from Aβ_{1-40} group, $p<0.05$ (Two-way ANOVA followed by Newman-Keuls post hoc test).

mRNA levels of BDNF

Increased BDNF mRNA expression was observed in both cortex (Figure 4A) and hippocampus (Figure 4B) when animals are treated with atorvastatin or infused with A$\beta_{1-40.}$ However, mice treated with atorvastatin plus Aβ_{1-40} (Ator+Aβ group) showed a reduction in BDNF mRNA levels to control levels in the cortex (Figure 4A). In the hippocampus, BDNF mRNA levels are also increased by Ator+Aβ treatment (Figure 4B).

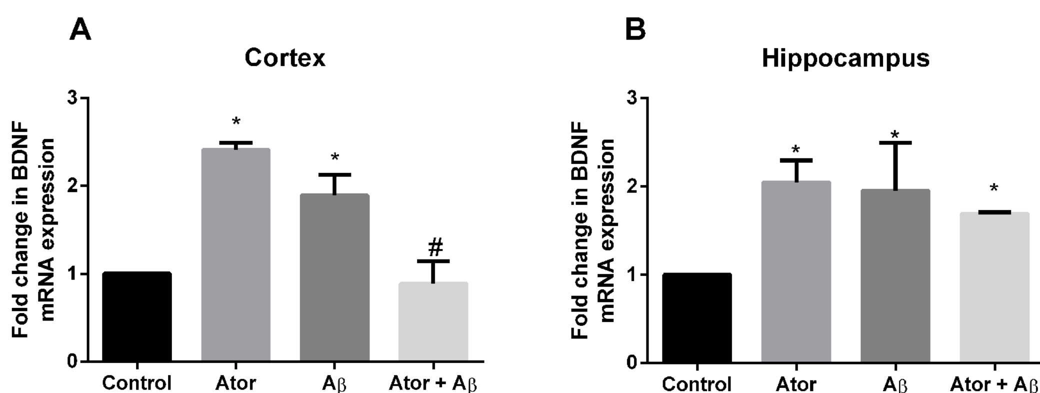

Figure 4: Evaluation of BDNF mRNA levels in cortex and hippocampus from mice subjected to atorvastatin pretreatment and i.c.v. infusion of Aβ_{1-40}. The animals received a 7-day pretreatment with atorvastatin (10 mg/kg, p.o.) or saline and a single i.c.v. infusion of Aβ_{1-40} (400 pmol/mouse) or PBS. The tissue samples were obtained 24 h after Aβ_{1-40} infusion. BDNF mRNA expression were evaluated in cortex (A) and hippocampus (B) regions. Results are presented as means ± S.D.
* indicates significant difference from control group, $p<0.05$, # indicates significant difference from Aβ_{1-40} group, $p<0.05$ (Two-way ANOVA followed by Newman-Keuls post hoc test).

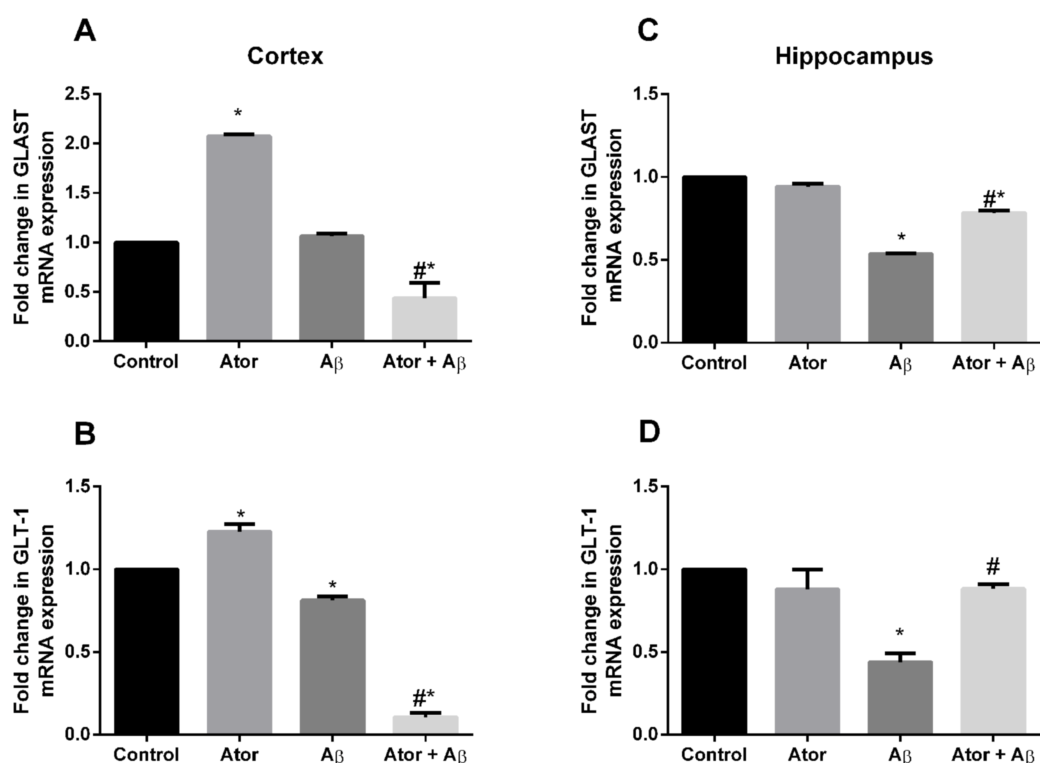

Figure 5: Evaluation of GLAST and GLT-1 mRNA levels in cortex and hippocampus from mice subjected to atorvastatin pretreatment and i.c.v. infusion of Aβ_{1-40}. The animals received a 7-day pretreatment with atorvastatin (10 mg/kg, p.o.) or saline and a single i.c.v. infusion of Aβ_{1-40} (400 pmol/mouse) or PBS. The tissue samples were obtained 24 h after Aβ_{1-40} infusion. Graphs represent the GLAST and GLT-1 mRNA expression in the cortex (A and B) and hippocampus (C and D), respectively. Results are presented as means ± S.D.
* indicates significant difference from control group, $p<0.05$, # indicates significant difference from Aβ_{1-40} group, $p<0.05$ (Two-way ANOVA followed by Newman-Keuls post hoc test).

mRNA levels of GLAST and GLT-1

Glutamate transporters GLAST and GLT-1 mRNA expression in the cerebral cortex of mice showed significant increase in atorvastatin treated group (Figure 5A and 5B). Infusion of Aβ_{1-40} did not change the basal (control) gene expression of GLAST, although it promoted reduction of GLT-1 mRNA. The presence of both treatments (Ator+Aβ) decreased both GLAST and GLT-1 mRNA bellow control levels.

In the hippocampus Aβ$_{1-40}$ decreased GLAST (Figure 5C) and GLT-1 (Figure 5D) mRNA levels. Pretreatment with atorvastatin for 7 days was capable to prevent the effect promoted by Aβ$_{1-40}$ infusion in GLT-1 mRNA (Figure 5D) and partially in GLAST mRNA expression (Figure 5C).

Discussion

We previously demonstrated that after 14 days of Aβ$_{1-40}$ infusion, mice displayed impaired learning, loss of short-term memory and some cellular dysfunctions related to AD. Atorvastatin treatment was able to prevent cognitive deficits, cellular death, oxidative stress and neuroinflammation promoted by Aβ [6,15]. Thus, in the present study we evaluated putative gene expression alterations in an early phase of Aβ$_{1-40}$- induced toxicity (24 h). We analyzed gene expression of proteins related to AD progression in the cortex and hippocampus of mice, two brain regions extremely affected by this pathology, and the effects promoted by the pretreatment with the neuroprotective agent atorvastatin.

Previous studies have shown dysfunction in the glutamatergic neurotransmission in experimental models of AD, such as reduction of basal levels of glutamate, as well as its astrocytic transporters and glutamate uptake [15,18]. It is feasible that alterations in glutamate transport may interfere with glutamate receptors expression. The NMDA receptor is a heterotetramer composed of a homodimer of GluN1 and dimers of GluN2A, 2B, 2C, 2D, or 3A, 3B subunits [19]. NMDA receptor is anchored in the membrane by interaction with PSD-95 and its activation permeates calcium influx. Our data in hippocampus region corroborate with the idea that Aβ toxicity may lead to dysfunction in glutamatergic neurotransmisson, more specifically in the transcription of the canonical NMDA receptor GluN1 subunit, whereas atorvastatin prevents the reduction promoted by Aβ. However, in the cortex Aβ infusion showed no alteration in NMDA-GluN1 mRNA in relation to control group, while the animals pretreated with atorvastatin that also received Aβ showed a reduction in this gene expression.

Similar results were obtained for PSD-95 mRNA expression, the post-synaptic anchoring protein to NMDA receptors, in both analyzed structures. The diminished levels of PSD-95 mRNA promoted by Aβ in the hippocampus may aggravate the expression of NMDA receptors on cell surface resulting in loss of synaptic plasticity [20]. Atorvastatin pretreatment blocked the reduction of PSD-95 mRNA by Aβ. Other statins like simvastatin has been implicated in the increase of NMDA receptor binding in various brain regions, including hippocampus [21]. These data demonstrate that GluN1 and PSD-95 mRNA expression respond in a similar way to Aβ and atorvastatin in both brain structures, although different effects were observed between the hippocampus and cerebral cortex as a response to the early events of Aβ toxicity.

The NOS1 enzyme is the main producer of nitric oxide (˙NO) in the brain and one of the NOS isoforms that display aberrant patterns of expression in AD, altering intracellular signaling and redox homeostasis, leading to nitrosative stress damage [7]. The activation of NOS1 via NMDA receptors (NMDAR) requires interaction with the scaffold protein PSD-95, which forms an NMDAR/PSD-95/NOS1 complex. NMDAR/PSD-95/NOS1 association plays an important role in several normal neuronal functions including synaptic plasticity and learning and memory, as well as pathophysiological disorders of the brain [22]. Our data show a reduction in NOS1 mRNA levels in atorvastatin, Aβ and atorvastatin+Aβ groups in the hippocampus. This effect promoted by atorvastatin was expected since it is well known that atorvastatin have antioxidants properties [6,23]. However it was also demonstrated an involvement of ˙NO improving the effect of atorvastatin in the cognitive consolidation phase [24]. Conversely, atorvastatin was not able to alter NOS1 gene expression in the frontal cortex. Interestingly, the reduction achieved by the infusion of Aβ in our data conflicts with the literature, since most studies show increased gene expression and activity of NOS1 in AD models [25,26]. This might be explained by the early period that mRNA levels were evaluated after Aβ infusion.

Activation NMDAR is also involved with the release of BDNF [27], which in turn is suggested to regulate NOS1 activity [28]. In the brain, BDNF is more active in areas related to learning and memory, such as the hippocampus and cortex, involved in the process of growth, differentiation and survival of neurons and synapses [29]. Our findings demonstrated an increase in BDNF mRNA in both brain regions in animals groups treated with atorvastatin and Aβ only. In the cortex, pretreatment with atorvastatin following Aβ infusion did not change in relation to control levels opposite to the data obtained from hippocampus, which also increased the levels of this gene. One might expect a reduction in the levels of BDNF mRNA by the Aβ toxicity, as studies demonstrated that BDNF can improve learning and memory deficits and exert neuroprotection in some models of AD [30]. However, other studies showed variations in BDNF mRNA levels depending of the mouse strain used [31]. Moreover we have to keep in mind that after the transcription, BDNF must undergo processing until its mature form that leads to neuroprotection. The proBDNF form has been described as an inductor of learning and memory deficits in aged mice possible through synergy toxicity with Aβ [32,33]. From another point of view, the decrease and increase of NOS1 and BDNF mRNA levels, respectively, by Aβ$_{1-40}$ might be interpreted as a compensatory response to its toxicity.

The clearance of glutamate from the synaptic cleft is another important mechanism to prevent excitotoxicity caused by a dysfunction in the glutamatergic neurotransmission. We analyzed the gene expression of GLAST and GLT-1, the main glial transporters involved in this neurotransmitter clearance. The infusion of Aβ reduced the GLT-1 mRNA in both regions studied and GLAST-1 mRNA in the hippocampus. The pretreatment with atorvastatin prevented this reduction promoted by Aβ in the hippocampus region,

demonstrating a possible neuroprotection mechanism to diminish glutamate-induced excitotoxicity. Region and age specific changes in glutamate transport, as well as in others mechanisms related to AD were already demonstrated in literature [6,34]. Furthermore, evidence shown that Aβ reduce protein expression of both glial transporters in the hippocampus after 16 days and a treatment with atorvastatin can revert this effect [15] which is consistent with our findings.

In summary, atorvastatin increases mRNA levels from GluN1, PSD-95, BDNF and glutamate transporters in the cerebral cortex. In the hippocampus atorvastatin did not alter glutamatergic genes (GluN1, PSD-95, GLT-1 and GLAST), but it reduced NOS1 and increased BDNF gene expression, what may be related to its neuroprotective action. Regarding Aβ$_{1-40}$ only effect, it seems it presented a more diffuse effect over the cortical gene expression, as it reduced the gene expression of glutamatergic proteins and NOS1, but increased BDNF mRNA levels, what might be interpreted as a compensatory response to Aβ$_{1-40}$ toxicity, more evident in the hippocampal region. Finally, in mice receiving atorvastatin+Aβ$_{1-40}$, it is almost always observed an opposite effect from the observed in Aβ$_{1-40}$-treated groups, reinforcing the data on the neuroprotective effect of atorvastatin against Aβ-induced toxicity even in early molecular alterations.

Acknowledgement

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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