

Original Article

Differential peroxisome proliferator activated receptors activity in a rodent model of amyotrophic lateral sclerosis

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Received January 9, 2015; Accepted February 24, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder that is characterized by the irreversible loss of corticospinal neurons and motor neurons. Recent studies has demonstrated an anti-inflammatory activity for the Peroxisome Proliferator-Activated Receptor (PPARs) agonists, which in ALS have been able to decrease the production of proinflammatory genes, including cytokines and chemokines. The comprehension of the molecular mechanisms that are responsible for their neuroprotective activity of PPARs could possibly lead to identify new targets for unprecedented therapeutic approaches. Using a PPRE-Luc; hSOD1-G93AALS transgenic mice we investigated the PPAR transcriptional activity over the course of ALS pathogenesis. The analysis of the enzymatic activity of luciferase in the spinal cord and the brain areas of PPRE-Luc; hSOD1-G93A mice showed an abrupt increase of PPAR activity at the end stage of the disease in the spinal cord, which was not shared by the peripheral organs. Furthermore, it was not dependent on the metabolic modifications induced from the starvation that the animals experienced during the last days of their life when they are almost completely paralyzed. Analysis of the nuclear translocation of PPAR α , PPAR β/δ and PPAR γ in the spinal cord of hSOD1-G93A mice with an ELISA-based Transcription Factor Assay showed that the overall nuclear presence of the different isoforms of PPARs did not change during the course of the disease. Our results indicate that the increase in PPAR activity at the end stage of the disease could represent a compensatory mechanism aimed at counteracting the intense neurodegenerative process which takes place at this time.

Keywords: Amyotrophic lateral sclerosis, ALS, peroxisome proliferator-activated receptor, PPAR, hSOD1-G93A, PPRE-Luc

Introduction

During the last few years, a growing number of studies have demonstrated an anti-inflammatory activity for the Peroxisome Proliferator-Activated Receptor (PPARs) agonists, which in several pathological instances have been able to decrease the production of proinflammatory genes, including cytokines and chemokines [1-3].

Based on these observations, the therapeutic impact of PPARs agonists has been more recently studied also in chronic neurodegenerative disorders characterized by neuroinflammatory processes, like Multiple Sclerosis, Alzheimer's disease, Parkinson's disease and

Amyotrophic Lateral Sclerosis (ALS). In animal models of different neurodegenerative diseases, PPARs agonists proved to be efficacious in attenuating the manifestations of the pathology, and this effect was ascribed to their ability in reducing the production of proinflammatory mediators [4-9]. Particular attention was focused on the PPAR γ agonist Pioglitazone because of its capacity to penetrate the blood brain barrier. This compound was shown to be beneficial in many animal models of neurodegenerative diseases [10-15], including mice that reproduce several features of ALS. Two independent groups demonstrated a neuroprotective activities of Pioglitazone on the hSOD1-G93A transgenic mouse model of ALS. In these

studies, administration of Pioglitazone, before the onset of the symptoms, improved the motor performance and reduced the weight loss, attenuated motor neuron death and increased the survival delaying the onset. These effects were associated to reduced microglial activation and gliosis in the spinal cord as well as decreased production of proinflammatory mediators like iNOS, NF- κ B and COX2.

As yet, different mechanisms of PPARs activation have been described, some of which directly related to gene transcription and other interfering with the activity of other transcription factors [16], but the signaling pathways involved and the specific events responsible for their neuroprotective activity have not been clearly elucidated. The comprehension of the molecular mechanisms that are responsible for their neuroprotective activity could then possibly lead to identify new targets for unprecedented therapeutic approaches.

hSOD1-G93A mice ubiquitously express a high copy number of the glycine to alanine base pair mutation at the 93rd codon of the cytosolic human Cu/Zn superoxide dismutase gene [17]. These mice develop progressive motor neuron disease that recapitulates in many aspects the human pathology: the symptoms manifest around 90 days of age when the animals show hindlimb weakness, the development of tremors and loss of weight [17, 18]. Motor functions progressively decrease leading to complete paralysis and death at around 130-150 days of age. At the histopathological level, the phenotype is associated to selective degeneration of spinal motor neurons, protein inclusions in surviving motor neurons and significant loss of myelinated axons originating from the ventral horns of the spinal cord. Female hSOD1-G93A are infertile, so the transgenic mice hSOD1-G93A are maintained in hemizyosity in the mixed background C57Bl6/SJL by mating of hSOD1-G93A mice with wild type C57Bl6/SJL females.

On these bases, the aim of the current work was to study the transcriptional activity of the PPAR systems *in vivo*, in the central nervous system, throughout the course of ALS with the aim of clarifying the stage of the disease at which the activity of this class of receptors becomes relevant for the pathology.

Materials and methods

Animals

Wild-type (WT) and hSOD1-G93A mice [B6.Cg-Tg(SOD1-G93A)1Gur/J] [17] were bred at the First Affiliated Hospital of Harbin Medical University from founding stock imported from Jackson Laboratories (Bar Harbor, ME, USA). Mice were group housed ($n > 3$) in a 12-h light, 12-h dark cycle (on at 0600 h and off at 1800 h). Room temperature was maintained at $22 \pm 2^\circ\text{C}$, and mice had free access to food (19.6% protein, 4.5% fat) and water, unless otherwise mentioned. In line with ethical requirements, symptomatic hSOD1-G93A mice were provided with wet food and water on the floor of their cages. Procedures were approved by Institutional Animal Care and Ethics Committee at the First Affiliated Hospital of Harbin Medical University and were performed in accordance with international guidelines. The transgenic mouse line PPRE-Luc mice [B6.D2-Tg (MAR-PPRE-tk-luc-MAR) Top/J] [19] were obtained from Charles River, Guangdong, China. Heterozygous hSOD1-G93A female mice were crossed with homozygous PPRE-Luc male mice to obtain hSOD1-G93A $^{+/-}$; PPRE-Luc $^{+/-}$ and hSOD1-G93A $^{-/-}$; PPRE-Luc $^{+/-}$ mice which were used for subsequent experiments.

Mice genotyping

Tail biopsies (1-2 mm) were lysed in 100 μl of lysis buffer (10 mM TRIS/HCl pH 9.0; 50 mM KCl; 0.45% Nonidet P40; 0.45% Tween 20; 0.1 mg/ml PK (proteinase K)) for 12 hours. The samples were then heated at 95°C for 10 min to inactivate the PK and then centrifuged at 13200 rpm for 20 min to precipitate the remaining tissue. The supernatant containing the genomic DNA was then used for subsequent PCR analysis. The primers used for hSOD1-G93A amplification were: 5' SOD: 5' CAT CAG CCC TAA TCC ATC TGA 3' and 3' SOD: 5' CGC GAC TAA CAA TCA AAG TGA 3'. IL-2 primers used in the same reactions as internal control were the following: 5' IL: 5' CTAGGC CACAGAA-TTGAAAGATCT 3' and 3' IL: 5' GTAGGTGG-AAATTCTAGCATCATCC 3'. The reaction mixture was the following: 0.4 μM primers, 200 μM dNTPs, 1X DNA Pol buffer and 32 U/ml DyNAzyme II DNA polymerase. 0.5-1 μl of the supernatant of the lysed tail were added to 25 μl of reaction mixture. After 35 cycles (30 sec

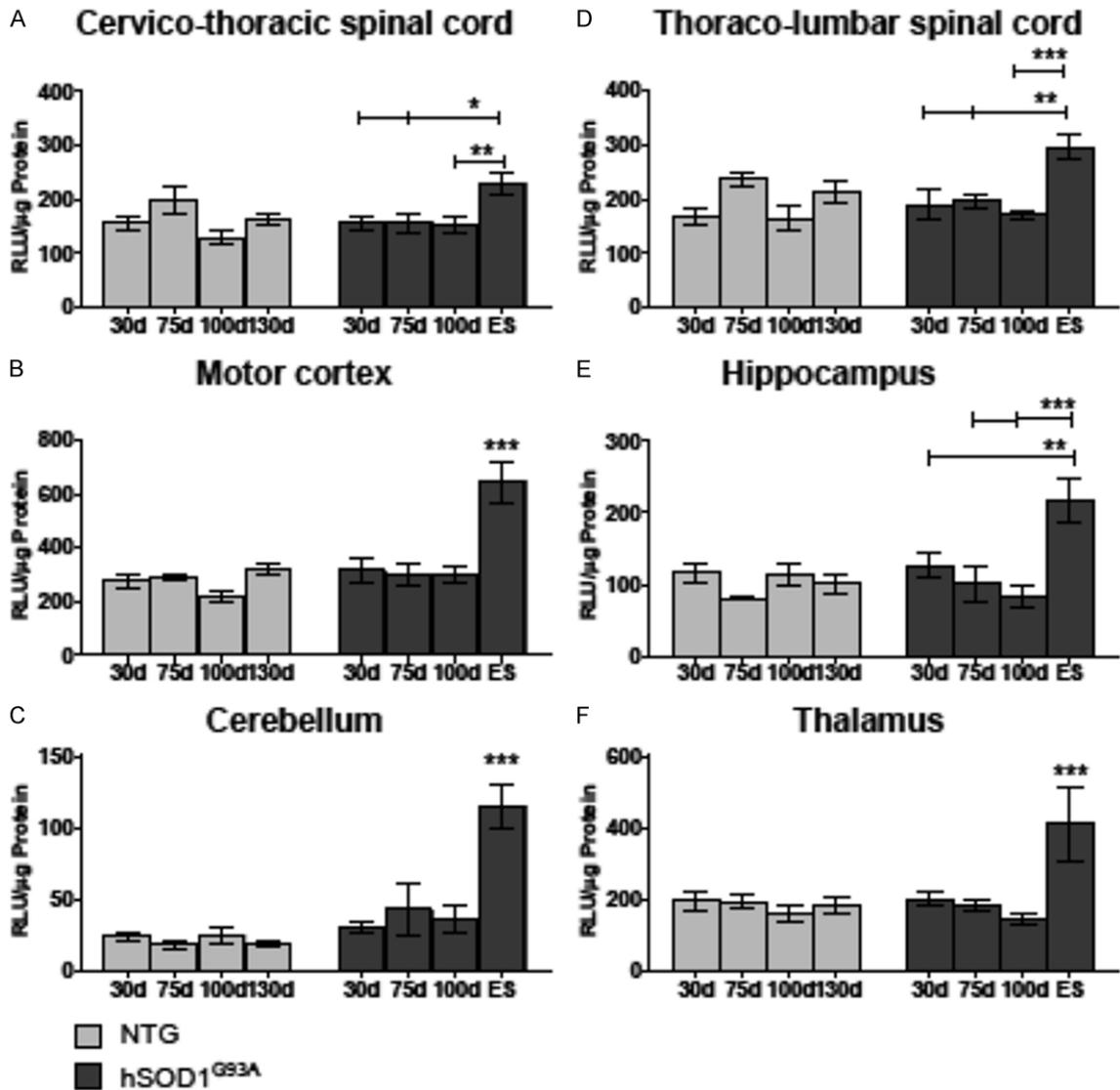


Figure 1. Evaluation of PPARs activity in the different CNS areas of hSOD1-G93A mice at the different stages of the pathology. The cervico-thoracic (A) and thoraco-lumbar (D) spinal cord, the motor cortex (B), the cerebellum (C), the hippocampus (E) and the thalamus (F) of female *PPRE-Luc*^{+/-} mice transgenic or not for the mutated (G93A) human SOD1 were harvested at the critical stages of the pathology: 30, 75, 100 days (d = days), end stage (ES). The luciferase activity was normalized to the total protein content. The data are expressed as RLU (Relative Luminescence Unit)/ μ g of total proteins \pm SEM. Two-way analysis of variance (ANOVA) followed by Bonferroni Post Test was used to compare the means of the different stages in the transgenic and non-transgenic groups. *P < 0.05, **P < 0.01, ***P < 0.001.

at 95°C, 30 sec at 60°C, and 30 sec at 72°C), the products were analyzed on 2% agarose gels stained with ethidium bromide in TAE buffer (0.04 M Trizma Base; 0.02 M acetic acid; 1 mM EDTA; 0.5 μ g/mL ethidium bromide). The amplicons of hSOD1-G93A and IL-2 were fragments of 250 bp and 320 bp long, respectively. The amplification of genomic DNA from transgenic animals produced both the bands, while the PCR of genomic DNA from non-transgenic ani-

mals resulted only in the 320 bp IL-2 band (data not shown).

Luciferase enzymatic assay

To quantify the luciferase activity in tissue extracts from luciferase reporter mice, Mice were anesthetized with sodium pentobarbital (i.p., 32 mg/kg) before the collection of tissue samples. The tissues of interest were collected

PPAR in a rodent model of ALS

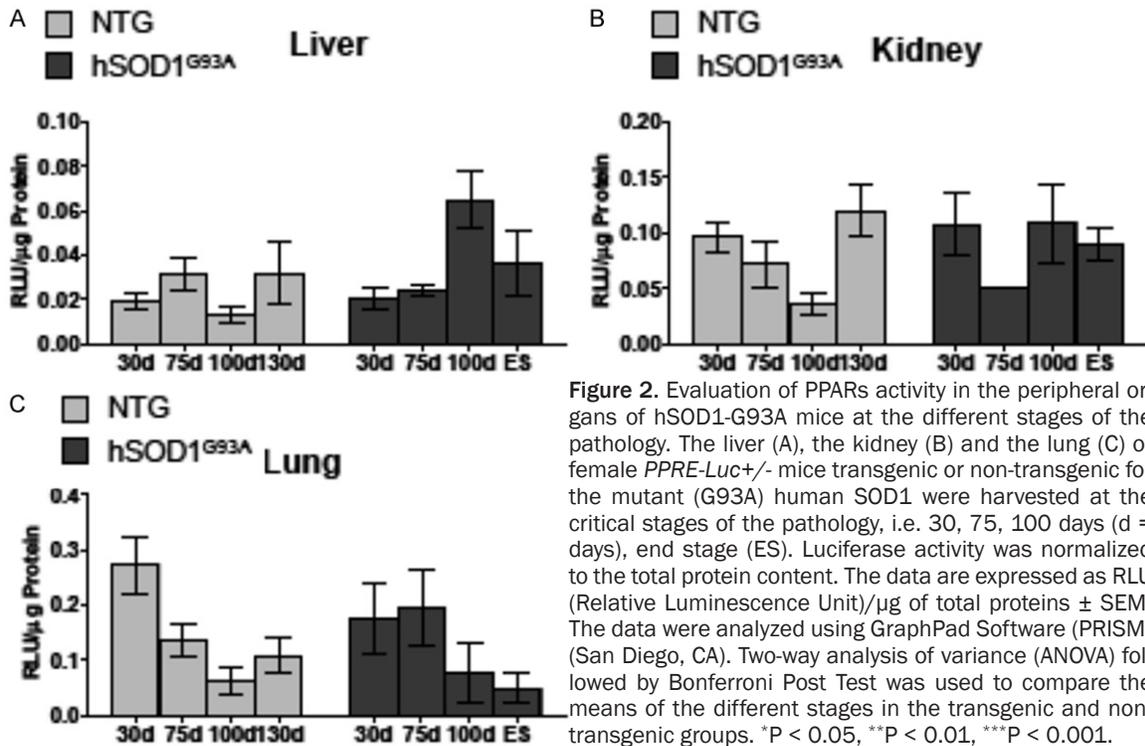


Figure 2. Evaluation of PPARs activity in the peripheral organs of hSOD1-G93A mice at the different stages of the pathology. The liver (A), the kidney (B) and the lung (C) of female *PPRE-Luc*^{+/-} mice transgenic or non-transgenic for the mutant (G93A) human SOD1 were harvested at the critical stages of the pathology, i.e. 30, 75, 100 days (d = days), end stage (ES). Luciferase activity was normalized to the total protein content. The data are expressed as RLU (Relative Luminescence Unit)/ μ g of total proteins \pm SEM. The data were analyzed using GraphPad Software (PRISM) (San Diego, CA). Two-way analysis of variance (ANOVA) followed by Bonferroni Post Test was used to compare the means of the different stages in the transgenic and non-transgenic groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and immediately frozen in dry ice and stored at -80°C . Tissues were homogenized in 200 μL of lysis buffer (100 mM KPO_4 pH 7.8, containing 1 mM dithiothreitol; 4 mM EGTA; 4 mM EDTA and 0.7 mM phenylmethylsulfonyl fluoride) using a TissueLyser (QIAGEN GmbH, Hilden, Germany) with 3 cycles of 10 sec with a 22 Hertz frequency. After one cycle of freezing-thawing to promote the breaking of cellular membranes the samples were centrifuged at 5900 rpm for 30 min at 4°C . The supernatants containing the protein extracts were then collected and used for subsequent luciferase enzymatic assay. 20 μL of tissue extract were added to the wells of a white 96 multiwell at 37°C , then the plate was positioned inside the luminometer (LUMAT LB 9501 Berthold) that injected sequentially 100 μL of a solution containing luciferine (470 μM luciferin (Promega); 20 mM Tricine (Gibco); 0.1 mM EDTA (Merk); 1.07 mM $(\text{MgCO}_3)_4\text{Mg}(\text{OH}) \times 25\text{H}_2\text{O}$ (Sigma); 2.67 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ (Merk) in bdH_2O pH 7,8 with 33.3 mM DTT (Boheringer Mannheim) and 530 μM ATP (Boheringer Mannheim) in each well. The luminometer detected the photon emission (RLU, Relative Luminescence Unit) coming from the enzymatic reaction between the luciferase inside the samples and the luciferin in the buffer 10 sec after having injected the substrate in

each well of the plate. The values of RLU were then normalized on the total protein content quantified with the Bradford Protein Assay.

Nuclear and cytoplasmic protein fractionation from spinal cord

Frozen spinal cords were weighed and cut in small pieces with a scalpel on ice. The tissue was then homogenized in 3 ml per gram of tissue of hypotonic lysis buffer (20 mM Hepes pH 7.5; 5 mM NaF; 10 μM Na_2MoO_4 ; 0.1 mM EDTA; 1 mM DTT; 0.01 % NP-40) and incubated on ice for 15 min. The samples were then centrifuged at $850 \times g$ for 10 min at 4°C . The supernatant obtained from this centrifugation represents the cytosolic fraction which was transferred to another tube. The pellet was resuspended in 500 μL of hypotonic lysis buffer without DTT and NP-40. The samples were then incubated on ice for 15 min, then 50 μL of 10% NP-40 were added to the samples and the tubes were centrifuged at $14000 \times g$ for 30 sec at 4°C . The supernatant obtained was the cytosolic fraction and was transferred to the tube together with the fraction previously isolated. The cytosolic fraction then was stored at -80°C . The pellet was then resuspended in 50 μL of extraction buffer (10 mM Hepes pH 7.9; 0.1 mM EDTA; 1.5

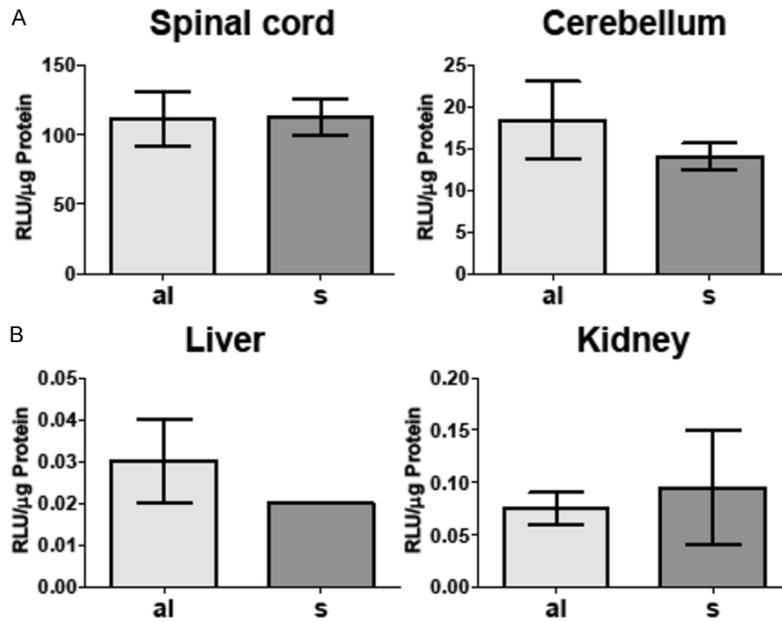


Figure 3. Evaluation of PPARs activity in the spinal cord and cerebellum (A) and liver and kidney (B) of *PPRE-Luc*^{+/-} female mice after starvation. Female *PPRE-Luc*^{+/-} mice were fed ad libitum (al) or starved (s) for 48 h. Luciferase activity was normalized on the total protein content. The data are expressed as RLU (Relative Luminescence Unit)/μg of total proteins ± SEM. The data were analyzed using GraphPad Software (PRISM) (San Diego, CA). T test was used to compare the means of the different stages in the transgenic and non-transgenic groups. *P < 0.05.

mM MgCl₂; 420 mM NaCl; 0.5 mM DTT; 0.5 mM PMSF; 1 μg/mL Pepstatin A; 1 μg/mL Leupeptin; 10 μg/mL Aprotinin; 20 mM NaF; 1 mM β-Glycerophosphate; 10 mM Na₃OV₄; 25% Glycerol). The samples were vortexed at high speed and incubated at 4°C in slow agitation for 30 min. The samples were then centrifuged at 14000 x g for 10 min at 4°C, the supernatant was the nuclear fraction and was stored at -80°C. The cytoplasmic and nuclear extracts were then quantified with the Bradford Protein assay and used for subsequent assay.

Quantitative assay for PPAR DNA binding

Nuclear presence of PPARα, PPARβ/δ and PPARγ was assayed using an enzyme linked immunosorbent assay-based PPARα,-δ,-γ Transcription Factor Assay Kit (Cayman Chemical). Nuclear proteins were extracted from spinal cords of hSOD1-G93A mice and non-transgenic littermates according to the manufacturer's instructions and protein concentration was determined by the Bradford method using BSA as standard, as previously described. A double-stranded DNA sequence containing the PPAR response element was linked to the

bottom of wells (96-well plate). PPARs within the nuclear fraction bound specifically to this sequence and isoforms were detected using primary antibodies directed against the individual PPARs. Clarified cell lysates were supplied for each PPAR isoform and acted as effective positive controls for PPAR DNA binding. Specificity of binding was also demonstrated using wells with no nuclear protein added and wells with positive control and an excess of consensus oligonucleotide (WT oligonucleotide) added which competes with the oligonucleotide bound to the wells. In these wells, no binding was detected. Binding activity was measured at 450 nm (minus the blank). The data obtained were then expressed like medium optical density (O.D.)/μg of proteins.

Statistical analyses

Statistical analysis data were obtained by means of analysis of variance (ANOVA) using GraphPad Software (PRISM) (San Diego, CA). Difference between groups was determined by Bonferroni comparison; a P < 0.05 was considered statistically significant.

Results

Increasing evidence demonstrates the PPARγ plays neuroprotective and anti-inflammatory roles in various neurodegenerative diseases, including ALS [20]. Furthermore several indications suggest that PPARs are implicated in a number of signaling transduction pathways potentially involved in neuronal activity and survival, suggesting that dysfunction of these receptors may influence the neuronal pathophysiology. However the specific mechanisms by which PPARs exert their neuroprotective roles remain to be further elucidated.

To investigate the activity of PPARs in the central nervous system during the progression of

PPAR in a rodent model of ALS

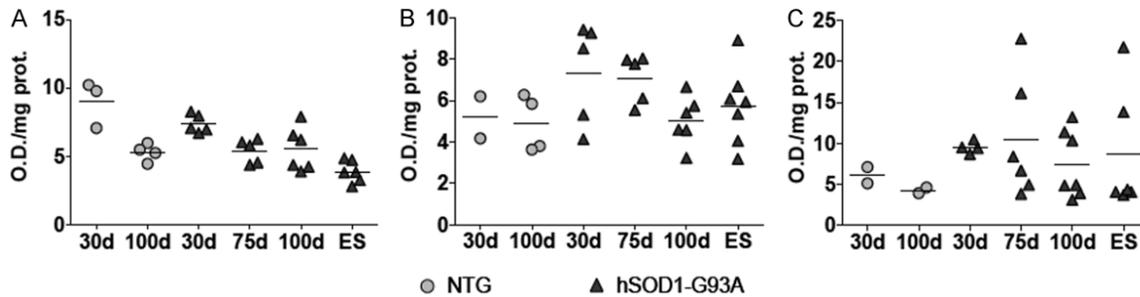


Figure 4. Evaluation of the nuclear presence of PPAR α (A), PPAR β/δ (B), and PPAR γ (C) in the spinal cord of hSOD1-G93A and non-transgenic mice. Spinal cords of female *PPRE-Luc*^{+/-} mice transgenic or non-transgenic for the mutant (G93A) human SOD1 were harvested at the critical stages of the pathology, i.e. 30, 75, 100 days (d = days), end stage (ES). The nuclear fraction was utilized for an ELISA-based assay. The data were expressed as optical density (O.D.)/mg of total proteins \pm SEM. The data were analyzed using GraphPad Software (PRISM) (San Diego, CA). Two-way analysis of variance (ANOVA) followed by Bonferroni Post Test was used to compare the means of the different stages in the transgenic and non-transgenic groups.

ALS, we decided to analyze the transcriptional activity of these receptors in the spinal cord of hSOD1-G93A mice during the course of the disease taking advantage of the reporter *PPRE-Luc* mouse line [19], in which the luciferase reporter gene is expressed under the control of a promoter responsive to PPARs. Homozygous female *PPRE-Luc* mice (*PPRE-Luc*^{+/+}) were crossed with heterozygous male *hSOD1-G93A*^{+/-} animals, obtaining hemizygous *PPRE-Luc*^{+/-} transgenic or non-transgenic for the human mutated SOD1 (hSOD1-G93A).

The animals were sacrificed at the critical stages of the pathology: 30 days (pre-symptomatic stage), 75 days (intermediate stage in which the motor neuron starts to detach from the motor plaque), 100 days (onset of the symptoms) and end stage, i.e. when the mice are unable to right themselves within 30 sec when being placed on their side. Since the end stage of hSOD1-G93A mice is around 120-140 days of age, non-transgenic mice were sacrificed at 30 days, 75 days, 100 and 130 days as controls. The cervico-thoracic and thoraco-lumbar spinal cord, the motor cortex, the hippocampus, the thalamus and the cerebellum were collected. We collected also the liver, the kidney and the lung to verify possible influences by the disease in the peripheral organs, which are not involved in the pathological process. The harvested tissues were immediately frozen at -80°C until subsequent analysis. To reduce the variability linked to the gender we focused our studies only on female mice. Five mice per each stage of the pathology were analyzed.

The data showed that, in non-transgenic mice, PPAR activation is fairly constant throughout the progression of the pathology in all CNS areas taken into consideration, with the exception for non-statistically significant differences. The hSOD1-G93A mice showed a similar trend in the cervico-thoracic spinal cord (**Figure 1A**), motor cortex (**Figure 1B**) and cerebellum (**Figure 1C**) until the onset of the pathology at 100 days, then luciferase activity increased abruptly and significantly at the end stage ($P < 0.001$) (**Figure 1D**). The hippocampus (**Figure 1E**) and thalamus (**Figure 1F**) showed non-significant decreases in luciferase activity from 30 to 100 days, then the signal increased at the end stage ($P < 0.001$). The luciferase activity in the peripheral tissues was variable but we never observed the increase in luciferase activity observed in the CNS of hSOD1-G93A mice at the end stage (**Figure 2**). The CNS-specific increase in PPAR activity in the hSOD1-G93A mice was a novel finding that warranted further investigation.

PPARs are known to be widely involved in the regulation of multiple metabolic processes. Therefore, we decided to verify whether the starvation that the hSOD1-G93A mice experience in the last period of their life, due to dysphagia and complete paralysis which prevents them from reaching for food and water, could influence PPARs activity. The effect of starvation was analyzed on selected CNS areas (spinal cord and cerebellum) and peripheral organs (liver and kidney) of *PPRE-Luc*^{+/-} mice. *PPRE-Luc* female mice were divided into two groups:

one group was fed *ad libitum* (*ad libitum*, al) while the other group was deprived of food and water for 48 h (starvation, s). The animals were sacrificed and the spinal cord, the cerebellum, the liver and the kidney were collected and immediately stored at -80°C for subsequent luciferase enzymatic assay. The results show that the luciferase activity in the spinal cord and cerebellum (**Figure 3A**) is constant between the two experimental groups. In the liver of starved animals, luciferase activity decreases slightly compared to controls, while no significant variability was detected in the kidneys (**Figure 3B**).

The results obtained led us to conclude that the starvation does not influence PPAR activity neither in the peripheral organs of *PPRE-Luc+/-* mice nor in the CNS areas analyzed, particularly the spinal cord which is the tissue primary compromised in ALS. On these bases, we concluded that the increase in PPAR activity detected in the *PPRE-Luc+/-; hSOD1-G93A* female mice was not dependent on nutritional defects.

PPARs activation implies their translocation into the nucleus where they bind to the responsive elements in the promoter of target genes and regulate gene transcription. To evaluate if the nuclear translocation of PPAR α , PPAR β/δ and PPAR γ is modulated during the progression of the disease and if an increased nuclear translocation could be responsible for the increase in PPARs transcriptional activity at the end stage of the disease, we decided to quantify the amount of PPAR α , PPAR β/δ and PPAR γ in the nuclear fraction of spinal cord homogenates from *hSOD1-G93A* mice and non-transgenic littermates at the different stages of the pathology, using an ELISA-based Transcription Factor assay specific for each isoform of PPARs. Female *hSOD1-G93A* and non-transgenic littermates were sacrificed at 30, 75, 100 days or at the end stage and the spinal cords were collected. We found that the presence of PPAR α in the nucleus undergoes a progressive non-significant decrease in the spinal cord of *hSOD1-G93A* mice during the course of the disease and did not mimic the increase at the end stage seen with the luciferase assay (**Figure 4A**). The nuclear presence of PPAR β/δ decreased at the onset of symptoms, i.e. 100 days of age, and then increases but not significantly at the end stage (**Figure 4B**). The assay on the nuclear presence of PPAR γ shows a decrease until 100

days of age and then a non-significant increase at the end stage (**Figure 4C**).

Discussion

The vast majority of neurodegenerative disorders are adult-onset, incurable diseases. Understanding the pathogenetic mechanisms underlying these disorders and finding molecules apt to correct such processes are, therefore, among the fundamental topics of biomedical research. The need of effective therapy is also an urgent social need, especially in western countries, where the incidence of these conditions is growing up as a consequence of the aging of the populations and the increased lifespan.

Amyotrophic Lateral Sclerosis is one of the most common adult-onset neurodegenerative diseases characterized by progressive degeneration of upper and lower motor neurons leading to paralysis and death due to respiratory failure within 3-5 years from the onset. The incidence and prevalence of ALS are 1-2 and 4-6 per 100,000 each year, respectively, with a lifetime ALS risk of 1/600 to 1/1,000 [21]. Only one drug, riluzole, has proved effective in extending the lifespan of patients with ALS, but only by 3-6 months [22, 23]. For this reason the development of effective therapies for this pathology is highly invocated, but to date all attempts to develop novel treatments have failed. In this context, two recent reports on the neuroprotective activity of the PPAR γ agonist Pioglitazone in ALS mice result of considerable interest: in these studies, two independent groups demonstrated that Pioglitazone, an agent which is currently used in therapy for the treatment of type II diabetes, is neuroprotective in a mouse model of Amyotrophic Lateral Sclerosis, the *hSOD1-G93A* transgenic mice. Pioglitazone treatment started before the appearance of the symptoms, improved the motor performance and reduced the weight loss, attenuated motor neuron death and increased the survival. In addition, Pioglitazone reduced microglial activation and gliosis in the spinal cord, decreasing the production of pro-inflammatory mediators, such as iNOS, NF- κ B and COX2 [12, 14]. While the activity of the PPARs has been extensively characterized in peripheral organs, due to their well-known involvement in different metabolic pathways, the functions of the different isoforms in the central nervous system

have been investigated only in the last few years [4, 20]. Nevertheless, the beneficial properties of the PPARs towards the diseases of the CNS has recently gained more and more consideration based on the anti-inflammatory and neuroprotective activities recently demonstrated in a variety of animal models of neuroprotective disease, like Multiple Sclerosis, Parkinson's disease, Alzheimer's disease and Amyotrophic Lateral Sclerosis [11, 12, 14, 15, 24-27].

On this ground, we decided to investigate the transcriptional activity of PPARs in the central nervous system of the hSOD1-G93A mouse line, a well characterized animal model of Amyotrophic Lateral Sclerosis, with the aim of identifying the stage of the disease at which the activity of PPARs becomes relevant to the pathology. To this end, we took advantage of the transgenic mouse PPARE-Luc, in which the reporter gene luciferase is expressed under the control of a promoter responsive to PPARs [19]. Thus, we crossed the PPARE-Luc mice with the hSOD1-G93A animals to obtain mice that are heterozygous for the PPARE-Luc transgene and heterozygous or null for the hSOD1-G93A transgene. These mice represent an invaluable tool to investigate the transcriptional activity of PPARs during the progression of the disease, because luciferase activity is taken as a surrogate of PPAR activity and can be more easily measured. The analysis of the enzymatic activity of luciferase in the spinal cord and the brain areas of PPARE-Luc; hSOD1-G93A mice shows an abrupt increase of PPAR activity at the end stage of the disease in the spinal cord, which is the organ principally involved in the pathology, and in all the brain areas analyzed. We demonstrated that this phenomenon clearly depends on the pathology because it is not shared by the peripheral organs (e.g. kidney and liver). Furthermore, it is not dependent on the metabolic modifications induced from the starvation that the animals experience during the last days of their life when they are almost completely paralyzed and, thus, unable to reach food and water. We suggest that the increase in PPAR activity at the end stage of the disease could represent a compensatory mechanism aimed at counteracting the intense neurodegenerative process which takes place at this time. We subsequently decided to further investigate this mechanism by identifying the isoform(s) responsible for the increase of PPARs activity at the last stage of the disease

and the cell type(s) involved. We analyzed the nuclear translocation of PPAR α , PPAR β/δ and PPAR γ in the spinal cord of hSOD1-G93A mice with an ELISA-based Transcription Factor Assay. The results obtained from these experiments showed that the overall nuclear presence of the different isoforms of PPARs does not change during the course of the disease.

Taken together, these data revealed that the worsening of the pathology did not cause an increased PPAR translocation into the nucleus. These assays were performed on lysates from the entire spinal cord and, therefore, they did not provide any information on the different neural cell types; the increase of PPARs activity at the end stage of the pathology could be due to an enhanced presence of the PPARs only in selected cell types. Furthermore ligand-dependent effects derived from the interaction of PPARs with cofactors and regulatory molecules cannot be excluded. Both the aforementioned warrants further investigation.

Disclosure of conflicts of interest

None.

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