Activation of PPARγ by 12/15-lipoxygenase during cerebral ischemia-reperfusion injury

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Received July 17, 2014; Accepted October 20, 2014

DOI: 10.3892/ijmm.2014.1998

Abstract. Peroxisome proliferator-activated receptor γ (PPARγ) expression and activity are increased in brain ischemic injury and its agonists have shown potential for brain injury protection. The influence of 12/15-lipoxygenase (12/15-LOX) on the activity of PPARγ in oxygen-glucose deprivation (OGD) and ischemia-reperfusion (I/R) was investigated. A middle cerebral artery occlusion/reperfusion model with Sprague Dawley (SD) rats was established. For I/R intervention, the rats were treated with the 12/15-LOX-derived product 12-hydroxyeicosatetraenoic acid (12-HETE) for 30 min before cerebral artery occlusion. Primary cortical neurons from SD rats were used to establish an OGD cell model. 12-HETE or a 12/15-LOX antisense oligonucleotide (asON-12/15-LOX) was added to OGD-treated neurons. Western blots, immunofluorescence and enzyme-linked immunosorbent assays detected protein. Reverse transcription-polymerase chain reaction analyzed the expression of the PPARγ target genes. PPARγ-DNA binding activity was determined by peroxisome proliferator responsive element luciferase reporter vectors. 12/15-LOX total protein increased significantly with I/R, and expression of 12-HETE was also upregulated. 12-HETE treatment increased PPARγ protein expression and inhibited inducible nitric oxide synthase protein expression, which was upregulated with I/R. PPARγ nuclear protein and 12/15-LOX total protein expression in OGD-treated neurons increased significantly. 12-HETE treatment increased the expression of PPARγ nuclear protein, upregulated the mRNA levels of PPARγ target genes (lipoprotein lipase and acyl-CoA oxidase) and enhanced PPARγ-DNA binding activity. asON-12/15-LOX treatment inhibited 12/15-LOX and PPARγ protein expression and lipoprotein lipase mRNA. Cerebral I/R injury in rats and OGD treatment in neurons promoted 12/15-LOX expression, and 12-HETE activated PPARγ. Therefore, PPARγ can be activated by the 12/15-LOX pathway during cerebral I/R injury.

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Key words: 12-hydroxyeicosatetraenoic acid, peroxisome proliferator-activated receptor γ, oxygen-glucose deprivation, peroxisome proliferator responsive element

Introduction

Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor that controls multiple cellular metabolic processes and is a member of the nuclear hormone receptor protein family involved in the regulation of multiple physiological and pathological metabolic processes, such as lipid and glucose metabolism regulation *in vivo* and inflammation regulation (1). PPARγ is mainly expressed in monocytes/macrophages and fat cells, and low levels of PPARγ can be detected in the central nervous system. Recent studies have revealed that brain ischemic injury promoted the expression and activity of PPARγ (2) and that a PPARγ agonist can protect against brain ischemic injury through PPARγ activation (3,4).

12/15-lipoxygenase (12/15-LOX) is a fatty acid dioxygenase that can oxidize arachidonic acid into 12-hydroxyeicosatetraenoic acid (12-HETE) in mammalian cells. *In vitro* studies of non-neuronal cells revealed that 12-HETE can activate PPARγ (5). However, no studies have explored the influence of the 12/15-LOX pathway and 12-HETE on PPARγ activity in neurons damaged by ischemic injury. The present study investigated the changes in 12/15-LOX and 12-HETE levels and their impact on PPARγ activation in rat cortical neurons treated with oxygen-glucose deprivation (OGD). Simultaneously, antisense oligonucleotide technology was used to explore the influence of 12/15-LOX inhibition on PPARγ expression and activation to investigate the regulatory effect of 12/15-LOX pathway on PPARγ. The aim of the present study was to understand these mechanisms. The results may assist in the understanding of brain ischemic injury and help develop treatments in the future.

Materials and methods

Animal grouping and drug administration. All the animal experiments were carried out according to an institutionally approved protocol, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University (Tianjin, China).

Healthy adult male Sprague Dawley (SD) rats weighing 280-330 g were purchased from the Laboratory Animal Center at the Academy of Military Medical Sciences. All the rats consumed water freely but were fasted for 12 h before surgery. The rats were randomly allocated into three groups and were treated as follows: i) 12 rats in the sham-operation group were used for brain tissue homogenates for further analysis. ii) 6 rats in the ischemia-reperfusion (I/R) group were used for tissue sections and 12 rats were used for brain tissue homogenates for further analysis. iii) 12 rats in the I/R intervention group were used for brain tissue homogenates. 12-HETE was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA) and dissolved in phosphate-buffered saline (PBS) at 1 mg/ml. In the I/R intervention group, rats received stereotactic injections of 15 ml 12-HETE 30 min before ischemia induction, whereas the rats in the sham-operation group or I/R group received equal volumes of PBS.

Stereotactic intracerebroventricular injection. Rats were anesthetized with 10% chloral hydrate 30 min before the establishment of the middle cerebral artery occlusion/reperfusion (MCAO/R) model. Subsequently, 15 ml 12-HETE or an equal volume of PBS was injected into the right lateral ventricle at 0.8 mm posterior to bregma, 1.3 mm right lateral to midline and 3.5 mm deep into the subdural surface. Injection was performed at a rate of 1 μl/min and finished in 15 min. The needle was retained for 15 min and withdrawn at a rate of 1 mm/min to prevent overflowing of 12-HETE or PBS.

Establishment of MCAO/R model. The rat cerebral artery was occluded with thread for 60 min and reperfusion was performed for 24 h. In the sham-operation group the common carotid, external carotid and internal carotid arteries were exposed and separated by surgery without occlusion.

Primary culture of fetal rat cortical neurons and neuronal cell identification. Day 18-21 embryonic Sprague Dawley (SD) rats were sacrificed following anesthesia with ice and 75% ethanol and the cerebral cortex was isolated under sterile conditions. Pia mater and blood vessels were removed under a dissecting microscope. The cerebral cortex were cut into pieces and digested with 0.125% trypsin at 37˚C for 20 min. Subsequently, 20% fetal bovine serum (FBS) was added to stop digestion and the cells were triturated with a Pasteur pipette and filtered with a 200-mesh cell strainer to collect single-cell suspensions. The cells were counted with a hemocytometer. Cells were seeded into Petri dishes were pre-coated with 0.1 mg/ml L-polylysine at a final density of 10^5-10^6/ml. Primary cortical neurons were incubated at 37˚C in a 5% CO_2 humidified atmosphere and high glucose Dulbecco's modified Eagle's medium/nutrient mixture F-12 supplemented with 10% FBS, 10% horse serum, 1% glutamine and 1% penicillin-streptomycin was used as culture medium. The medium was changed to neurobasal medium (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 2% B27 following cell attachment (3 to 6 h after seeding). Half the volume of neurobasal medium was refreshed every third day. Neuronal-specific marker microtubule-associated protein 2 (MAP2) was stained at day 9 and >95% cells were MAP2 positive.

Preparation of the neuronal model of oxygen-glucose deprivation (OGD) and 12-HETE or 12/15-LOX antisense oligonucleotide (asON-12/15-LOX) treatment. Day 9 cortical neurons were randomly divided into the control, OGD-treated and intervention groups. The medium of the OGD-treated neurons was changed to neurobasal medium without glucose (Invitrogen Life Technologies) and the neurons were incubated in a pre-adjusted tri-gas incubator (37˚C, 95% N_2, 5% CO_2, <1% O_2). After 3 h incubation, neurons were cultured in the normal medium at the normal conditions for 24 h and were collected for further analysis. The 12-HETE intervention group was treated with 1 μM 12-HETE 30 min before OGD treatment and the 12-HETE concentration was maintained during the whole culture process. The asON-12/15-LOX intervention group was treated with 4 μM asON 48 h before OGD treatment and maintained during the whole culture process. The same volume of dimethyl sulfoxide (DMSO) was added into the OGD-treated group. The sequences of the antisense oligonucleotides used in the study are as follows: Antisense, 5'-CTC-AGG-AGG-GTG-TAA-ACA-3' (6); sense, 5'-TGT-TTA-CAC-CCT-CCT-GAG-3'; and scramble, 5'-AAG-ATT-GCG-GCG-CGA-CGA-TGA-3'.

Analysis of relative OGD-treated neuron survival rate by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Cortical neurons at day 9 were divided into 8 replicate blank groups (culture medium without neurons), control groups and OGD-treated groups. After treatment with OGD for 24 h, neurons were further cultured in normal medium and medium only was added into blank wells. Subsequently, 20 μl 5 mg/ml MTT was added into each well and incubated for 4 h. After discarding the medium, 100 μl DMSO was added, mixed thoroughly and incubated at 37˚C for 15 min. The optical density (OD) of each well was analyzed with an automatic plate reader and the absorbance at 570 nm was measured. The relative cell survival rate was calculated as follows: Relative cell survival rate = (OD OGD-treated group - OD blank group)/(OD control group - OD blank group). The median concentration was used for the model set.

Nuclear protein extraction. Nuclear proteins were isolated with a nuclear protein extraction kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions.

Western blotting. The primary antibodies were as follows: Mouse anti-rat PPARγ monoclonal antibody (1:200; Santa Cruz, Dallas, USA), rabbit anti-rat 12/15-LOX monoclonal antibody (1:1000; Cayman Chemical Co.); and related horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX, USA; 1:5000) were used as previously described (2).

ELISA analysis. The level of 12-HETE in neurons was determined with the 12-HETE ELISA kit (Assay Design, Ann Arbor, MI, USA) and performed according to the manufacturer's instructions.

Total RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR) amplification. Total RNA was extracted from the cells with TRIzol (Sigma, St. Louis, MO,

USA), precipitated with chloroform-isopropanol and quantified with absorbance at OD_{260}. cDNA was generated from 1.5 μg total RNA. PCR amplification was performed using a 20 μl reaction system, including 1 μl cDNA, 2 μl forward and reverse primers (5 μM), 200 μM dNTPs and 0.8 U Taq polymerase. Primers for lipoprotein lipase (*LPL*) were as follows: Forward, 5'-TTCCATTACCAAGTCAAGATTCAC-3'; and reverse, 5'-TCAGCCCGACTTCTTCAGAGACTT-3'. PCR amplification products were analyzed with 1.2% agarose gel electrophoresis and a gel imaging system (Tanon Science & Technology Co., Ltd., Shanghai, China) was used to quantify the band density. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as a loading control.

Construction of PGL3-PPRE and determination of luciferase activity. pGL3-basic (Promega, Madison, WI, USA) was used as the vector, which contains the luciferase mRNAs without promoter. Three peroxisome proliferator responsive element (PPRE) fragments were inserted upstream of the luciferase gene in the pGL3-basic vector to construct the pGL3-PPRE vector. Neurons were transfected with Lipofectamine 2000 (Invitrogen Life Technologies) and 24 h later, 1 μM 12-HETE was added, whereas in the control group, the same volume of DMSO was added. The cells were lysed 24 h later and luciferase analysis was performed according to the instructions of the luciferase assay system kit (Promega). A Safire2 basic plate reader from Tecan Australia Pty Ltd. (Melbourne, Australia) was used to measure the ODs of the cell lysates. The β-galactosidase enzyme assay system kit (Promega) was used to measure the β-gal activity to adjust the luciferase value for transfection efficiency. Adjusted fluorescence value = measured fluorescence value / β-gal value.

Immunofluorescence staining. Neurons were cultured on sterile slides precoated with poly-L-lysine. To perform immunofluorescence staining, neurons were fixed with 4% paraformaldehyde and blocked with serum for 30 min. Neurons were then incubated with a rabbit anti-rat 12/15-LOX polyclonal antibody (1:100; Cayman Chemical Co.) or anti-PPARγ antibody (1:50; Santa Cruz Biotechnology, Inc.) at 4˚C overnight. A tetraethyl rhodamine isothiocyanate-conjugated secondary antibody was added the next day and incubated in the dark at 37˚C for 1 h. Neurons were washed with PBS-Tween 20 and finally incubated with 4',6-diamidino-2-phenylindole at room temperature for 10 min to stain the cell nuclei. Slides were treated with mounting medium and analyzed with a Nikon Eclipse 80i microscope and Nikon DS-Ril camera (Nikon, Toyko, Japan).

Statistical analysis. Experimental data was exhibited as mean ± standard deviation. Statistical analysis was performed with SPSS 15.0 statistical package (SPSS Inc, Chicago, IL, USA) and an independent samples *t* test was performed for comparison between groups. A χ^2 test was used to analyze the difference in neuron survival rates between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Elevation of 12/15-LOX expression and activity induced by I/R injury. Western blots were performed to analyze the expression of 12/15-LOX in rat brain tissues from the I/R injury model. Significant upregulation of 12/15-LOX expression was induced by I/R injury (Fig. 1A). To explore the activity changes of 12/15-LOX, the level of its product, 12-HETE, was determined by ELISA and the results showed that I/R injury clearly induced the production of 12-HETE (Fig. 1B).

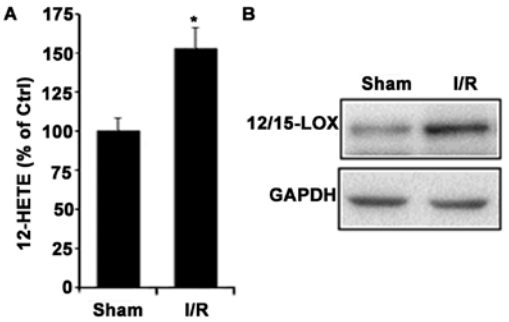

Figure 1. Analysis of 12/15-lipoxygenase (12/15-LOX) protein levels by (A) western blot and 12-hydroxyeicosatetraenoic acid (12-HETE) levels by (B) ELISA in brain tissues with ischemia-reperfusion injury. Sham, sham-operation group; I/R, ischemia-reperfusion group; *P<0.05, compared to the control group.

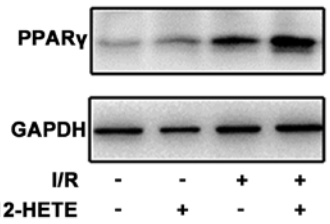

Figure 2. Analysis of peroxisome proliferator-activated receptor γ (PPARγ) expression in brain tissues with ischemia-reperfusion (I/R) injury by western blot. 12-HETE, 12-hydroxyeicosatetraenoic acid.

Induction of PPARγ expression by I/R injury and further upregulation of PPARγ expression with 12-HETE intervention. Western blotting showed that compared to the I/R group, I/R injury plus 12-HETE intervention markedly upregulated the expression of PPARγ (Fig. 2).

Suppression of inducible nitric oxide synthase (iNOS) expression by 12-HETE in rat brain tissues with I/R injury. Western blots were performed to explore the influence of 12-HETE intervention on iNOS expression and revealed an evident inhibition of iNOS expression (Fig. 3).

Upregulation of 12/15-LOX expression and 12-HETE generation by OGD treatment in neurons. The MTT assay revealed that the relative neuron survival rate decreased by 43.84±2.07% with OGD treatment for 24 h compared to the control group. The difference between the two groups was significant (P<0.05).

Western blots showed that the 12/15-LOX protein, the critical enzyme generating 12-HETE, increased significantly with OGD treatment compared with the control group (Fig. 4A) and immunofluorescence experiments confirmed that the upregulation of 12/15-LOX expression was induced by OGD (Fig. 4B).

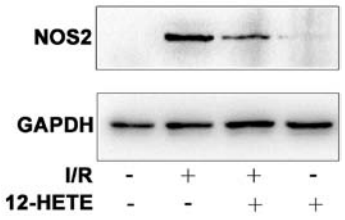

Figure 3. Analysis of inducible nitric oxide synthase (iNOS) expression in brain tissues with ischemia-reperfusion (I/R) injury by western blot. 12-HETE, 12-hydroxyeicosatetraenoic acid.

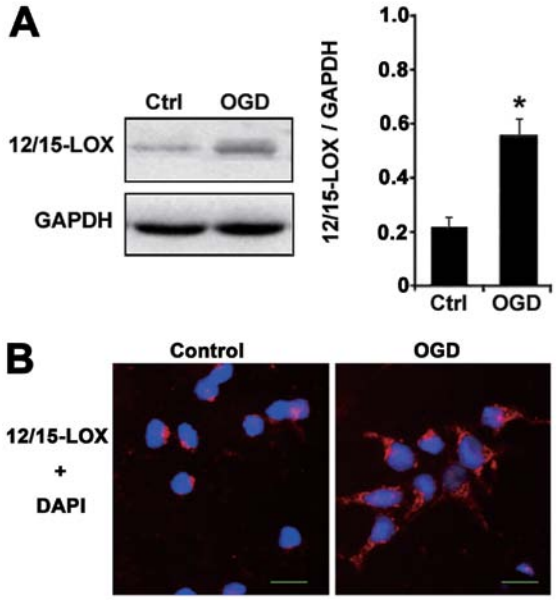

Figure 4. Analysis of the 12/15-lipoxygenase (12/15-LOX) protein levels in neurons by (A) western blot and (B) immunofluorescence. Nuclear staining was with 4',6-diamidino-2-phenylindole (DAPI; blue fluorescence), and 12/15-LOX staining was performed with a specific antibody (red fluorescence). Ctrl, control group; OGD, oxygen-glucose deprivation-treated group. *P<0.05, compared to control group.

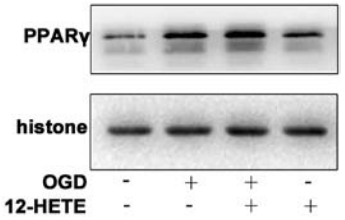

Figure 5. Analysis of peroxisome proliferator-activated receptor γ (PPARγ) expression in neuronal nuclei by western blot. OGD, OGD-treated group; 12-HETE, 12-hydroxyeicosatetraenoic acid intervention group.

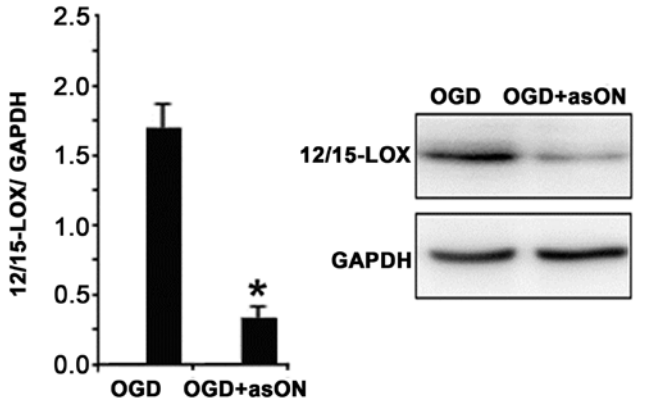

Figure 6. Analysis of 12/15-lipoxygenase (12/15-LOX) protein levels by western blot. OGD, oxygen-glucose deprivation-treated group; asON, 12/15-LOX antisense oligonucleotide intervention group. *P<0.05, compared to OGD-treated group.

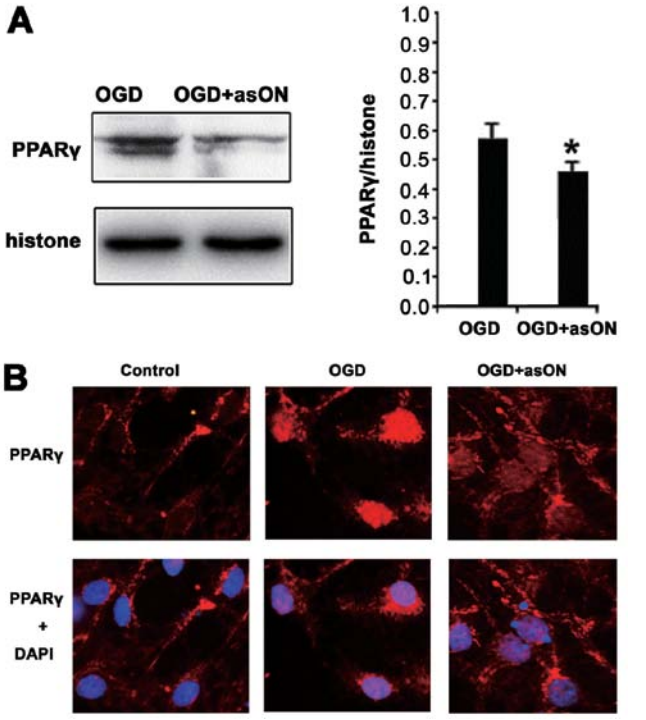

Figure 7. Analysis of peroxisome proliferator-activated receptor γ (PPARγ) nuclear protein expression in neurons by (A) western blot and (B) immunofluorescence. Nuclear staining was with 4',6-diamidino-2-phenylindole (DAPI; blue fluorescence), and PPARγ staining was performed with a specific antibody (red fluorescence). Overlapping signals (pink fluorescence) represent PPARγ in the nucleus. Control, control group; OGD, oxygen-glucose deprivation-treated group; asON, 12/15-LOX antisense oligonucleotide intervention group. *P<0.05, compared to OGD-treated group.

Stimulation of PPARγ nuclear protein expression by OGD treatment in neurons and upregulation of PPARγ nuclear protein expression by 12-HETE in OGD-treated neurons. Western blotting was used to determine the expression levels of PPARγ in the cell nucleus, and the results showed that PPARγ nuclear protein increased significantly in neurons with OGD treatment. PPARγ nuclear protein was also significantly elevated in the 12-HETE intervention plus OGD-treated group compared to the OGD-treated group (Fig. 5).

Blockade of 12/15-LOX expression by asON-12/15-LOX treatment in OGD-treated neurons. Compared to the OGD-treated group, asON-12/15-LOX treatment reduced the expression of 12/15-LOX significantly (Fig. 6). Whereas sense oligonucleotides or scramble oligonucleotides of 12/15-LOX did not change the expression of 12/15-LOX (data not shown).

Inhibition of PPARγ nuclear protein expression by asON-12/15-LOX treatment in OGD-treated neurons.

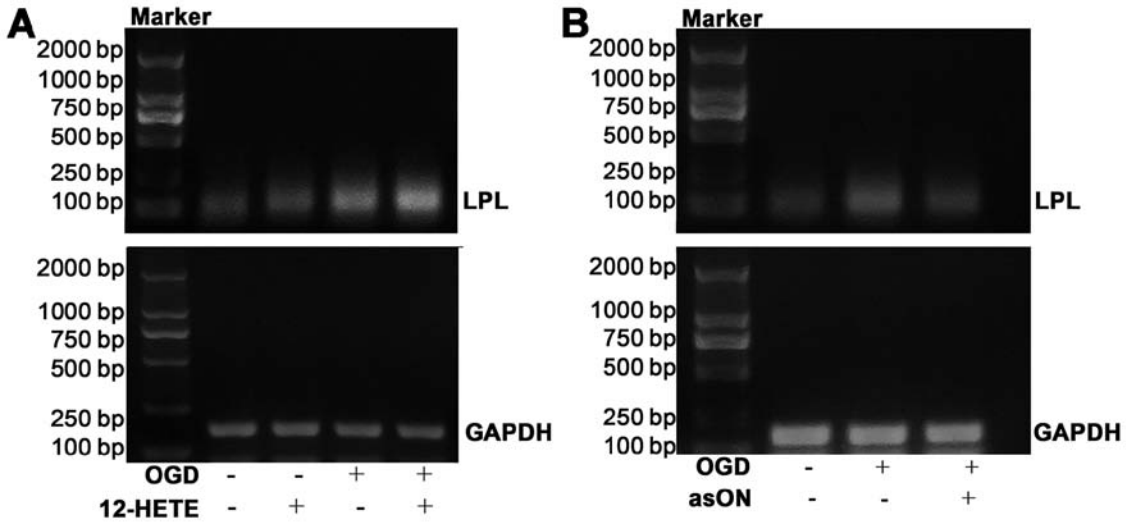

Figure 8. Expression of peroxisome proliferator-activated receptor γ (PPARγ) target genes determined by reverse transcription polymerase chain reaction (RT-PCR) following treatment with OGD and (A) 12-HETE or (B) asON. OGD, oxygen-glucose deprivation; 12-HETE, 12-hydroxyeicosatetraenoic acid; asON, 12/15-LOX antisense oligonucleotide.

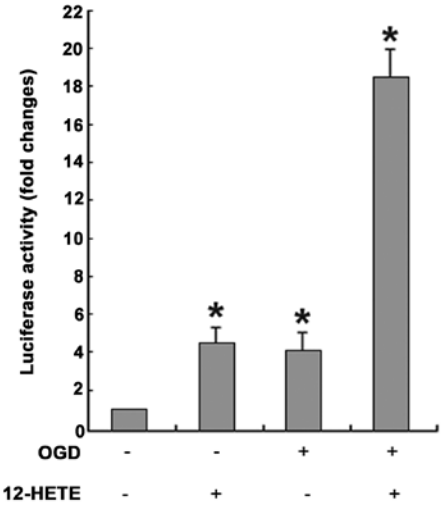

Figure 9. Peroxisome proliferator-activated receptor γ (PPARγ) binding to DNA measured by luciferase activity. *P<0.05, compared to control.

asON-12/15-LOX treatment inhibited the expression of PPARγ nuclear protein significantly compared to the OGD-treated group (Fig. 7A). Immunofluorescence analysis confirmed the results of the western blot, showing that the expression of PPARγ nuclear protein was upregulated with OGD treatment and the upregulation was inhibited by asON-12/15-LOX treatment (Fig. 7B).

Expression of PPARγ target genes by 12-HETE treatment and suppression of PPARγ target genes by asON-12/15-LOX treatment in OGD-treated neurons. RT-PCR was employed to analyze the expression of *LPL*, a PPARγ target gene, in OGD-treated neurons and to explore the impact of 12-HETE intervention on its expression. The results showed that 12-HETE intervention markedly increased the expression of *LPL* mRNA compared to OGD-treated neurons (Fig. 8A). However, treatment with asON-12/15-LOX clearly inhibited the expression of *LPL* in OGD-treated neurons (Fig. 8B).

Enhancement of PPARγ binding ability to DNA by 12-HETE treatment. Normal cultured cortical neurons were transfected with pGL3-PPRE and were subsequently treated with 12-HETE, OGD or OGD plus 12-HETE. After 24 h, luciferase activities in the treated neurons clearly increased compared to control neurons treated with DMSO (t=–10.753, –9.679 and –21.978, respectively, P<0.05). Luciferase activity increased similarly in 12-HETE- and OGD-treated neurons with 4-fold changes, but luciferase activity increased by 18-fold in neurons treated with OGD plus 12-HETE (Fig. 9). Furthermore, relative survival rates of neurons were measured with the MTT assay and the possibility that luciferase activity was enhanced by cell survival changes derived from 12-HETE or OGD treatment was excluded (data not shown). Only minor luciferase activity was detected in 12-HETE-treated neurons transfected with pGL3-Basic, whereas luciferase activity could not be detected in neurons transfected with mixtures without any plasmids or in neuron lysates.

Discussion

The aim of the present study was to investigate the influence of 12/15-LOX on the activity of PPARγ in ischemia reperfusion. This was of interest as this information may assist with future treatment of brain ischemic injury. PPARγ is a member of the nuclear receptor superfamily and a ligand-dependent transcriptional factor. PPARγ activation leads to its nuclear translocation in order to regulate the transcription of target genes. Numerous studies have reported that PPARγ expression and activity in brain tissues were induced by I/R injury (7-10), and that PPARγ activation reduced infarct volume and inhibition of inflammation mediators including intercellular adhesion molecule 1, interleukin-β, cyclooxygenase-2 and iNOS (3,4,11,12). Therefore, it has been proposed that induction of PPARγ expression and activation by brain ischemia is a protective response to damage. This protective response may be attributed to PPARγ activation induced by endogenous agonists following ischemia, but the detailed mechanisms remain elusive.

12/15-LOX, a member of lipoxygenase family, is a lipid peroxidase encoded by the *ALOX15* gene that can oxidize free polyunsaturated fatty acids and phospholipids in biological membranes to generate oxidative products. 12-HETE is the oxidative derivative of arachidonic acid catalyzed by 12/15-LOX. In brain tissues, 12/15-LOX is mainly expressed in neurons or certain astrocytes in the cerebral hemisphere, basal ganglia and hippocampus. 12-HETE is the major product of 12/15-LOX in the central nervous system (3). Previous studies revealed that in brain tissues, 12/15-LOX may exert its physiological functions through oxidative modifications of membrane structures and generation of mediators or signaling molecules with biological activities involved in synaptic transmission (13,14). *In vitro* studies confirmed that 12/15-LOX products, such as 13-hydroxyoctadecadienoic acid (13-HODE), 12-HETE and 15--HETE served as endogenous PPARγ agonists. Furthermore, higher expression levels of 12/15-LOX enhanced the transcriptional activation effect of PPARγ in specific cell types. For example, in monocytes, 12/15-LOX products interacted with PPARγ directly to stimulate the expression of cluster of differentiation 36 and upregulate PPARγ expression simultaneously (15). The induction of PPARγ and its target genes by IL4 in macrophages through the 12/15-LOX pathway has already been shown. Similarly, 13-HODE and 15-HETE PPARγ and increase the expression of *PPARγ* mRNA in human vascular smooth muscle cells (16) and colon tumor cell line (17). Furthermore, *in vivo* experiments have confirmed the regulatory effect of 12/15-LOX on PPARγ and in the mouse uterus, PPARγ activation can occur through the 12/15-LOX pathway to mediate the impact of 12/15-LOX on the pregnant uterus (5). These results indicate that elevation of PPARγ expression and activation could be induced by 12/15-LOX in multiple tissues and cells. Therefore, we speculate that 12/15-LOX has similar roles in the central nervous system.

In the present study, I/R injury has been demonstrated to induce the expression of 12/15-LOX and its product 12-HETE. A previous study implicated the upregulation of 12-HETE level with oxidative stress caused by I/R injury in brain tissues (18). However, other studies have shown that 12-HETE is involved in synaptic transmission as a second messenger in the central nervous system and that it also participates in a variety of physiological activities, including learning and memory (19,20). Notably, 12-HETE served as an endogenous agonist of PPARγ to modulate its activity (5).

PPARγ is a nuclear transcription factor and following activation it transports into the cell nucleus from the cytoplasm to regulate the expression of target genes. Therefore, the expression of PPARγ protein in the nucleus is associated with its activity status. Regulation of target gene expression by PPARγ is through PPARγ binding to a specific DNA element in the promoters of the target genes (peroxisome proliferator responsive element, termed PPRE) to promote target gene expression (21-23).

The present study revealed that 12-HETE intervention in rats with I/R injury elevated the expression of PPARγ total protein. Furthermore, treatment of OGD-damaged rat cortical neurons with 12-HETE induced the expression of PPARγ, enhanced its binding ability to DNA and promoted the expression of target genes, suggesting the stimulatory effect of 12-HETE on PPARγ activity. Several associated studies have reported an inhibitory effect of PPARγ on inflammation following ischemia. For instance, PPARγ agonists, including pioglitazone, rosiglitazone and troglitazone, reduced infarct volume, improved neuron functions, suppressed the expression of variant inflammatory mediators, reduced neutrophil infiltration, and inhibited the activation of microglias, macrophages and the inflammation-associated NF-κB pathway (4,10-12). Furthermore, other studies have reported that the neuroprotective effect of PPARγ is partially achieved through suppression of iNOS expression and activation (24-27). In accordance with these results, it was observed that elevated expression of iNOS in brain tissues with I/R injury was inhibited by 12-HETE intervention and PPARγ activity was stimulated simultaneously, indicating that 12-HETE-induced PPARγ activation inhibited inflammation responses, to achieve a neuroprotective effect.

Antisense oligonucleotides were also used to inhibit the expression of 12/15-LOX and revealed that PPARγ nuclear expression was negatively regulated by asON-12/15-LOX, confirming the regulatory effect of 12/15-LOX on PPARγ. Cell transfection reagents are often used to promote the delivery of antisense oligonucleotides into cells to enhance the inhibitory effect. However, cell transfection reagents often damage cells. As primary cultured neurons *in vitro* are vulnerable and prone to injury, the oligonucleotides were dissolved into the culture medium directly to treat neurons. This method inhibited the expression of 12/15-LOX significantly, indicating its feasibility. Previous studies have also proved the efficiency of such methods in sensory neuron treatment (6,28).

The present study has certain limitations. Rat-based models of oxygen-glucose deprivation and ischemia reperfusion were used, however, it would be noteworthy to observe if the role of 12/15-LOX can also be followed in human derived cells. A number of details of the mechanism of PPARγ protection remain to be revealed and therefore, further work is required prior to considering these results in terms of clinical therapy.

In conclusion, the level of the 12/15-LOX-derived product 12-HETE was significantly elevated in OGD-treated cortical neurons and confirmed the agonistic effect of 12-HETE on PPARγ. The expression of PPARγ nuclear expression could be blocked with 12/15-LOX inhibition in OGD-treated neurons. These results revealed that PPARγ is activated by the 12/15-LOX pathway in OGD-treated neurons and that PPARγ activation has a neuroprotective effect, indicating that it is a neuronal self-protective response to damage and injury.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant no. 81401023).

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