Research Article

PPAR-γ Impairment Alters Peroxisome Functionality in Primary Astrocyte Cell Cultures

Lorenzo Di Cesare Mannelli, Matteo Zanardelli, Laura Micheli, and Carla Ghelardini

Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino—(Neurofarba)—Sezione di Farmacologia e Tossicologia, Università di Firenze, Viale Pieraccini 6, 50139 Florence, Italy

Correspondence should be addressed to Lorenzo Di Cesare Mannelli; lorenzo.mannelli@unifi.it

Received 15 November 2013; Revised 4 January 2014; Accepted 7 January 2014; Published 4 March 2014

Copyright © 2014 Lorenzo Di Cesare Mannelli et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peroxisomes provide glial cells with protective functions against the harmful effects of H$_2$O$_2$ on neurons and peroxisome impairment results in nervous lesions. Agonists of the γ-subtype of the Peroxisome-Proliferator-Activated-Receptors (PPAR) have been proposed as neuroprotective agents in neurodegenerative disorders. Nevertheless, the role of PPAR-γ alterations in pathophysiological mechanisms and the relevance of peroxisome functions in the PPAR-γ effects are not yet clear. In a primary cell culture of rat astrocytes, their reversible PPAR-γ antagonist GW9662 concentration-dependently decreased the activity of catalase, the most important antioxidant defense enzyme in peroxisomes. Catalase functionality recovered in a few days and the PPAR-γ agonist rosiglitazone promoted reversal of enzymatic damage. The reversible antagonist G3335 reduced both the activity and expression of catalase in a rosiglitazone-prevented manner. G3335 reduced also the glutathione reductase expression, indicating that enzyme involved in glutathione regeneration was compromised. Neither the PPAR-α target gene Acyl-Coenzyme-A-oxidase-1 nor the mitochondrial detoxifying enzyme NADH:ubiquinone-oxidoreductase (NDUF53) was altered by PPAR-γ inhibition. In conclusion, PPAR-γ inhibition induced impairment of catalase in astrocytes. A general decrease of the antioxidant defenses of the cell suggests that a PPAR-γ hypofunction could participate in neurodegenerative mechanisms through peroxisomal damage. This series of experiments could be a useful model for studying compounds able to restore peroxisome functionality.

1. Introduction

Hydrogen peroxide (H$_2$O$_2$) is ascribed to Reactive Oxygen Species (ROS), although it has no unpaired electrons. It can be formed by the dismutation reaction of O$_2^−$ via the hydroperoxy radical. Although H$_2$O$_2$ is not harmful, its conversion, through the Fenton reaction catalyzed by metal ions, generates the hydroxyl radical (•OH), probably the most highly reactive and toxic form of oxygen [1–3]. Catalase is a heme-containing peroxisomal enzyme that breaks down hydrogen peroxide to water and oxygen and is a main antioxidant defense [4, 5]. De Duve and Baudhuin [6] first described a respiratory pathway in peroxisomes in which electrons removed from various metabolites reduce O$_2$ to H$_2$O$_2$, which is further reduced to H$_2$O. The high peroxisomal consumption of O$_2$, the demonstration of the production of H$_2$O$_2$, O$_2^−$, •OH, and recently of •NO [6–9], and the discovery of several ROS metabolizing enzymes in peroxisomes has supported the notion that these ubiquitous organelles play a key role in both the production and scavenging of ROS in the cell [1].

Together with oxygen metabolism, peroxisomes fulfill multiple tasks [10]. The functional relevance of these organelles is dramatically highlighted in the nervous system by peroxisomal disorders. Genetic diseases classified as peroxisome biogenesis disorders and single peroxisomal enzyme deficiencies imply severe demyelination, axonal degeneration, and neuroinflammation that result in a variety of neurological abnormalities [11–15]. On the other hand, peroxisomes have recently been involved in cell aging [16] and in the development and progression of specific degenerative diseases [14, 17–22].

Since a common feature of several neurodegenerative diseases is inflammation [23], several studies have pointed
to the potential use of agonists of the Peroxisome Proliferator Activated Receptor-γ (PPAR-γ). Increasing evidence demonstrates the neuroprotective effects of PPAR-γ agonists in a variety of preclinical models of neurological disorders such as Alzheimer’s disease [24–26], Parkinson’s disease [27], amyotrophic lateral sclerosis [28], Huntington’s disease [29], and ischemic damage [30]. Nevertheless, evidence of PPAR-γ impairment in the physiopathology of neurodegenerative diseases is lacking, as well as the effects induced by its hypofunctionality in the nervous system. The theoretical basis of a PPAR-γ therapeutic approach in neurodegenerative disorders is generally founded on the anti-inflammatory effect. A clear relationship with peroxisome impairments is not well established. Although PPARs can transactivate genes pivotal for the functionality of these organelles [31, 32], the role of peroxisomes in PPAR-γ agonist efficacy, or in PPAR-γ hypofunction, remains unclear.

By focusing on astrocytes, glial cells strongly implicated in several degenerative diseases [33–35], we aimed to characterize the relevance of peroxisome functionality in PPAR-γ-dependent cell signaling. We have evaluated the damage evoked by PPAR-γ antagonists in a primary cell culture by analyzing characteristic peroxisome enzymes.

2. Material and Methods

2.1. Astrocyte Cultures. Primary cultures of astrocytes were obtained according to the method described by McCarthy and De Vellis [36]. Briefly, the cerebral cortex of newborn (P1–P3) Sprague-Dawley rats (Harlan, Udine, Italy) was dissociated in Hank’s balanced salt solution containing 0.5% trypsin/EDTA and 1% DNase (Sigma-Aldrich, Milan, Italy) for 30 min at 37°C. The suspension was mechanically homogenized and filtered. Cells were plated in high-glucose DMEM with 10% FBS. Confluent primary glial cultures were used to isolate astrocytes, removing microglia and oligodendrocytes by shaking. The purity of astrocyte cultures was determined immunocytochemically by staining for GFAP (Dako, Glostrup, Denmark). Cells were fixed in 4% paraformaldehyde, then incubated with the antibody (1:200), and visualized using Alexa Fluor-conjugated secondary antibody (Life Technologies, Monza, Italy). Nuclei were stained with 4,6-diamidino-2-phenylindole dihydrochloride. 90% of cells in astrocyte cultures were GFAP-positive. Experiments were performed 21 days after cell isolation. Treatments with GW9662 (100mM) for 2 or 5 days. All compounds were purchased from Sigma-Aldrich (Milan, Italy). After incubation, cells were washed once with PBS and scraped with PBS on ice. Cells were then collected, subjected to a freeze-thaw cycle, and centrifuged at 11,000 × g for 10 min at 4°C. Catalase activity was measured in the supernatant by Amplex Red Catalase Assay Kit (Invitrogen, Monza, Italy) following the manufacturer’s instructions. Protein concentration was quantified by bicinchoninic acid assay (Sigma-Aldrich, Milan, Italy). Catalase activity for each sample was normalized to protein concentration. Control conditions in the absence of treatment were set as 100%. Basal catalase activity was not different on days 0 (48 h after plating), 2, 4, 7, or 10 of culturing.

2.3. Hydrogen Peroxide Levels. On day 21 of culture, astrocytes were plated in 6-well cell culture (5 · 10⁵/well; Corning, Tewksbury MA, USA) and experiments were performed 48 h after. After treatments with G3335 and rosiglitazone (2 and 5 days), cells were washed once with PBS and scraped with PBS on ice. Cells were then collected, subjected to a freeze-thaw cycle, and centrifuged at 11,000 × g for 10 min at 4°C. Supernatants were treated with sorbitol to convert peroxide to a peroxyl radical, which oxidizes Fe²⁺ into Fe³⁺. Then the reaction between Fe³⁺ and an equal molar amount of xylenol orange in the presence of acid was allowed to create a purple product. The absorbance was read at 595 nm (OxiSelect Hydrogen Peroxide Assay Kit, Cell Biolabs, San Diego, CA, USA).

2.4. Western Blotting Analysis. On day 21 of culture, astrocytes were plated in 6-well cell culture (5 · 10⁵/well; Corning, Tewksbury MA, USA) and experiments were performed 48 h after. Treatments with G3335 and rosiglitazone lasted 2 and 5 days. After incubation, cells were washed once with PBS and scraped on ice with lysis buffer containing 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, Complete Protease Inhibitor (Roche, Milan, Italy). Cells were then collected, subjected to a freeze-thaw cycle, and centrifuged at 11,000 × g for 10 min at 4°C; the supernatant was conserved. Astrocyte protein extract was quantified by bicinchoninic acid assay and 40 μg of each sample was resolved with 10% SDS-PAGE before electrophoretic transfer onto nitrocellulose membranes (Biorad, Milan, Italy). Membranes were blocked with 5% nonfat dry milk in PBS containing 0.1% Tween 20 (PBST) and then probed overnight at 4°C with primary antibody specific versus catalase (1:1000; 60 kDa; Novus Biological, Littleton, CO, USA), acyl-CoA oxidase I (ACOXI) (1:1000; 75 kDa; Santa Cruz Biotechnology, Santa Cruz, CA, USA), peroxisomal membrane protein of 70 kDa (PMP70) (1:1000; Abcam, Cambridge, MA, USA), glutathione reductase (1:1000; 65 kDa; Santa Cruz Biotechnology, Santa Cruz, CA, USA), NDUFS3, core subunit of Complex 1 NADH:ubiquinone oxidoreductase (1:1000; 30 kDa; Abcam, Cambridge, MA, USA), GAPDH (1:1000; 38 kDa; Cell Signaling, Boston, MA, USA), and β-Actin (1:1000; 42 kDa; Cell Signaling, Boston, MA, USA). Membranes were then incubated for 1 hour in PBST containing the appropriate horseradish peroxidase-conjugated secondary antibody.
Values are expressed as the mean ± S.E.M. percent of control of three experiments. Control catalase activity was arbitrarily set as 100%. *P < 0.05 in comparison to control conditions in the absence of treatment.

2.5. Statistical Analysis. Results are expressed as mean ± S.E.M. and analysis of variance (ANOVA) was performed. A Bonferroni significant difference procedure was used as post hoc comparison. All assessments were made by researchers blinded to cell treatments. P values of less than 0.05 were considered significant. Data were analyzed using the Origin 8.1 software (OriginLab, Northampton, MA, USA).

3. Results

The activity of the peroxisomal enzyme catalase was evaluated in astrocyte cell culture using a fluorometric assay. The irreversible PPAR-γ antagonist GW9662 reduced catalase activity in a dose-dependent manner over time. As shown in Figure 1, a 2-day incubation with 30 μM GW9662 decreased catalase activity to 81.4 ± 3.6% (control arbitrarily set at 100%), an effect comparable to that evoked by 100 μM H₂O₂ after 2 h incubation (data not shown). The enzymatic activity decreased to 69.8 ± 2.8% after a 5-day incubation in the presence of 30 μM GW9662 and to 32.7 ± 1.3% in the presence of 100 μM GW9662 (Figure 1).

The activity impairment induced by 100 μM GW9662 for 2 days (activity decreased to 61.0 ± 0.9%) was not prevented by the PPAR-γ agonist rosiglitazone (100 μM) (Figure 2(a)). Allowing a further 2-day incubation in the absence of GW9662 (day 4), catalase activity was fully restored (97.4 ± 6.7%) and in the presence of rosiglitazone was stimulated up to 136.1 ± 5.4%. On day 7, which was 5 days after GW9662 washout, activity was about 140% both in the absence and presence of rosiglitazone.

The strong catalase activity decrease induced by a 5-day incubation with 100 μM GW9662 (Figure 2(b)) was restored by a 2-day washout (day 7). The stimulatory effect of rosiglitazone was significant on day 10 (151.4 ± 6.0%; Figure 2(b)).

As shown in Figure 3, the reversible PPAR-γ antagonist G3335 (30 μM) induced catalase impairment (after a 2-day incubation 77.3 ± 4.1%; after 5 days 62.2 ± 5.3%) comparable to that evoked by GW9662 but this effect seems to be maximal since a higher concentration (100 μM) did not increase the damage (Figure 4). G3335-dependent catalase damage was prevented in the presence of 100 μM rosiglitazone both at 2 and 5 days (Figures 4(a) and 4(b)). Rosiglitazone was also able to improve catalase activity over 100% after a 2-day incubation (Figure 4(a)). The expression level of catalase was unaltered by 30 μM G3335 after a 2-day incubation. On the contrary, a 30% decrease induced by 5 days’ incubation of G3335 was fully prevented by 100 μM rosiglitazone (Figure 5). Catalase impairment was associated with a time-dependent increase of hydrogen peroxide levels (about 50 and 100% after 2- and 5-day incubation, resp., Figure 6). In the presence of rosiglitazone H₂O₂ levels were normalized.

G3335 did not alter PMP70 expression levels at both times evaluated (2 and 5 days, Figure 7(a)). G3335 did not alter ACOX1 expression levels at both times evaluated (2 and 5 days, Figure 7(b)). Expressions of the antioxidant enzyme glutathione reductase and Complex 1 NADH dehydrogenase were also measured to evaluate the protective response of astrocytes to the presence of G3335. The NDUFS3 subunit of the mitochondrial enzyme Complex 1 expression was not modified by G3335 (Figure 7(c)).

Glutathione reductase expression was progressively reduced by 30 μM G3335 over time. After 5 days’ incubation, protein levels decreased by about 40% (Figure 8), whereas 100 μM rosiglitazone prevented this effect (Figure 8).

4. Discussion

PPARs are members of the nuclear receptor superfamily, actively involved in immunoregulation through their ability to regulate membrane lipid composition, cell proliferation, sensitivity to apoptosis, energy homeostasis, and various inflammatory transcription factors, mainly through their transrepression capabilities [23]. Although all three subtypes of PPARs (α, β/δ, and γ [37]) have been implicated in brain damage, PPAR-γ is the most extensively studied [23, 38–40]. PPAR-γ agonists may ameliorate AD-related pathology and improved learning and memory in animal models and memory and cognition in AD patients [24–26]. Activation of
PPAR-γ by pioglitazone induces behavioral recovery associated with preservation of nigrostriatal dopaminergic markers and reduction of CD68-positive cells in Parkinsonian monkeys [27]. PPAR-γ agonists have beneficial effects in an experimental model of Huntington’s disease by interfering with the NF-κB signaling pathway [29]. Heneka et al. [30] showed that rosiglitazone delays neuronal damage by interfering with glial activations and increases anti-inflammatory cytokines in response to ischemic damage. On the other hand, there is scanty knowledge about the pathophysiological effects induced by PPAR-γ dysfunctions. In the present results a relationship between PPAR-γ inhibition in astrocytes and peroxisomal function impairment is shown. The irreversible PPAR-γ antagonist GW9662 concentration-dependently decreases catalase activity up to 30%. As expected, GW9662-dependent impairment is not prevented by the PPAR-γ agonist rosiglitazone. On the contrary, catalase functionality recovers in a few days in cell culture in the absence of GW9662, suggesting the plasticity of peroxisome in adverse conditions. Rosiglitazone stimulates the physiological restoration of the enzymatic damage leading to the hypothesis that PPAR-γ agonists may positively intervene in rescue signaling. The reversible antagonist G3335 reduces progressively catalase activity to 60% reaching a plateau for concentrations higher than 30 μM. Two days of incubation are needed for enzyme hypofunctionality, and decreased expression follows 3 days later. Both activity and expression reduction of catalase are prevented by rosiglitazone.

Catalase is the most important antioxidant defense enzyme in mammalian peroxisomes. In rodent liver peroxisomes, rough estimates indicate that each molecule of H₂O₂-producing oxidase possesses at least one molecule...
of catalase as a functional counterpart [41]. Considering that mammalian peroxisomes are densely populated by enzymes that form ROS (most of them are FAD- or FMN-dependent oxidases generating H$_2$O$_2$; [42]) it is not surprising that peroxisomes are well equipped with antioxidant defense systems composed mainly of enzymes involved in the decomposition of H$_2$O$_2$ [43]. Catalase impairment has been observed in neurodegenerative conditions [44] as well as in complex neurodevelopmental disorders such as autism spectrum, whose neurobiology is proposed to be associated with oxidative stress [45]. On the other hand, oxidative stress may result from an increase in ROS generation as well as from an impairment of catabolic phenomena. Alterations in consumer enzymes may vary the net rate between production and consumption and induce a release of ROS from the organelles to the cell [46]. H$_2$O$_2$, unlike O$_2^-$, is able to cross membranes and is free to leave the organelle and to induce cell damage [43]. On the contrary, catalase stimulation is protective against nervous injuries [47]. In the present results, a progressive increase of H$_2$O$_2$ parallels with catalase hypofunction.

Conversely, G3335-induced PPAR-γ block does not alter the expression of another major peroxisome protein, PMP70, a membrane protein possessing multiple peroxisome-targeting signals [48]. PMP70 is a half-type ABC-transporter [49] involved in the transport of long and branched chain acyl-CoA [50]. These data suggest lack of a relationship between PPAR-γ and PMP70 in astrocytes.

β-oxidation of a number of carbohydrates that cannot be handled by mitochondria is one of the most important metabolic reactions occurring in peroxisomes [51, 52] and this process also contributes to the formation of H$_2$O$_2$ [53, 54]. ACOX1 catalyzes the first and rate-limiting step of straight-chain fatty acid β-oxidation [55, 56]. ACOX1 is considered to be a PPAR-α target gene predictive of peroxisome proliferation [57, 58] but, given that PPAR subtypes recognize and activate gene expression through a common DNA binding site [59], ACOX1 could be regulated also by PPAR-γ. In the present results, G3335 does not modify the full length ACOX1 protein expression levels in astrocytes suggesting a specific regulation of PPAR-γ target genes.

To fulfill their functions, peroxisomes physically and functionally interact with other cell organelles, including mitochondria, the endoplasmic reticulum, and lipid droplets [1, 60]. It is well established that peroxisomes and mitochondria are metabolically linked in mammals [61]. Disturbance in peroxisomal metabolism triggers signaling/communication events that ultimately result in increased mitochondrial stress [62, 63]. To evaluate the effect of PPAR-γ inhibition on a characteristic enzyme of the detoxificant machinery of mitochondria we evaluated the expression levels of NDUFS3, a core subunit of Complex I, the first and largest of the four multiprotein complexes that constitute the mitochondrial respiratory chain involved in oxidative phosphorylation [64]. In particular, NDUFS3 primarily initiates the in vivo assembly of Complex I in the mitochondrial matrix [65]. Our results show that G3335 does not alter NDUFS3 expression in astrocytes, suggesting that these conditions are specific to peroxisomal damage. However, peroxisome impairment is enough to decrease glutathione reductase expression levels in a rosiglitazone-prevented manner. Glutathione reductase generates reduced glutathione, the main protector of the cell [66] and low levels of this enzyme may have implications for oxidative stress. Glutathione reductase has been described to be reduced in neurodegenerative diseases like PD [67], AD [44], adrenoleukodystrophy [66],

Figure 4: Catalase activity: effect of rosiglitazone on G3335-induced enzymatic impairment. Astrocytes (2 · 10^5 cells/well) were treated with the reversible PPARγ antagonist G3335 (30 μM) for (a) 2 days or (b) 5 days, in the absence or presence of the PPARγ agonist rosiglitazone (100 μM). Catalase activity was measured on days 0, 2, and 5. Values are expressed as the mean ± S.E.M. percent of control of three experiments. Control catalase activity (day 0) was arbitrarily set as 100%. ∗P < 0.05 in comparison to control conditions in the absence of treatment on day 0; †P < 0.05 in comparison to control on days 2 (a) or 5 (b).
Figure 5: Catalase expression: effect of G3335. Astrocytes \((5 \cdot 10^5 \text{ cells/well})\) were treated with the reversible PPAR\(\gamma\) antagonist G3335 \((30 \mu M)\) for 2 days or 5 days, in the absence or presence of the PPAR\(\gamma\) agonist rosiglitazone \((100 \mu M)\). Values are expressed as the mean \(\pm\) S.E.M. percent of control of three experiments. Control condition was arbitrarily set as 100%. \(^* P < 0.05\) in comparison to control conditions in the absence of treatment; \(^\wedge P < 0.05\) in comparison to G3335 treatment on day 5.

Figure 6: Hydrogen peroxide levels: effect of G3335. Astrocytes \((5 \cdot 10^5 \text{ cells/well})\) were treated with the reversible PPAR\(\gamma\) antagonist G3335 \((30 \mu M)\) for 2 days or 5 days, in the absence or presence of the PPAR\(\gamma\) agonist rosiglitazone \((100 \mu M)\). Values are expressed as the mean \(\pm\) S.E.M. percent of control of three experiments. Control condition was arbitrarily set as 100%. \(^* P < 0.05\) in comparison to control conditions in the absence of treatment; \(^\wedge P < 0.05\) in comparison to G3335 treatment on days 2 or 5.
Figure 7: (a) PMP70, (b) ACOX1, and (c) NDUFS3 expression: effect of G3335. Astrocytes (5 \times 10^5 cells/well) were treated with the reversible PPARγ antagonist G3335 (30 \mu M) for 2 days or 5 days, in the absence or presence of the PPARγ agonist rosiglitazone (100 \mu M). Values are expressed as the mean \pm S.E.M. percent of control of three experiments. Control condition was arbitrarily set as 100%.
and amyotrophic lateral sclerosis [68]. Astrocytes exert neuroprotective effects by providing neurons with substrates for antioxidants such as glutathione [69]. Astrocytes contain high levels of antioxidant molecules such as vitamins E and C and the antioxidant enzymes Mn- and Cu, Zn-superoxide dismutases (Mn- and Cu, Zn-SOD), catalase, and glutathione peroxidase, which play a major neuroprotective role against the deleterious effects of ROS [70, 71]. Although astrocytes are generally less susceptible to oxidative injury than neurons, there is strong evidence that oxidative stress also alters astrocyte functions [40, 72]. In particular, glial cells are extremely vulnerable to H$_2$O$_2$ and astrocytic apoptosis is observed in brain injuries caused by trauma and ischemia [73, 74] and in models of neuropathies [75]. Protection of astrocytes from oxidative attack appears essential to maintain cerebral antioxidant competence and to prevent neuronal damage as well as to facilitate neuronal recovery [76]. It has been shown that peroxisomes provide glial cells with neuroprotective and anti-inflammatory functions [77] and loss or impairment of peroxisomal function results in characteristic patterns of central nervous system lesions [11, 12]. This is best illustrated by pathomorphological examinations of the brain of patients (and mice) in which one or more peroxisomal functions are lost [12, 77–81].

5. Conclusion

In this report we highlight that the PPAR-γ block in astrocytes is strictly related to reduced catalase functionality and expression with a general decrease in antioxidant defenses of the cell. The relevance of the damage induced by PPAR-γ impairment suggests that hypofunctionality of this receptor in glial cells could be present in neurodegenerative diseases and participate in pathological mechanisms through peroxisomal damage. The present series of experiments could offer a useful model for the study of PPAR-γ agonists or, in general, compounds able to restore peroxisome functionality.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by the Italian Ministry of Instruction, University and Research.

References

[1] M. Schrader and H. D. Fahimi, “Peroxisomes and oxidative stress,” *Biochimica et Biophysica Acta*, vol. 1763, no. 12, pp. 1755–1766, 2006.
[2] L. Moldovan and N. I. Moldovan, “Oxygen free radicals and redox biology of organelles,” *Histochemistry and Cell Biology*, vol. 122, no. 4, pp. 395–412, 2004.
[3] P. Ježek and L. Hlavatá, “Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism,” *International
Journal of Biochemistry and Cell Biology, vol. 37, no. 12, pp. 2478–2503, 2005.

[4] C. D. Putnam, A. S. Arvai, Y. Bourne, and J. A. Tainer, "Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism," Journal of Molecular Biology, vol. 296, no. 1, pp. 295–309, 2000.

[5] S. K. Powers and M. J. Jackson, "Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production," Physiological Reviews, vol. 88, no. 4, pp. 1243–1276, 2008.

[6] C. De Duve and P. Baudhuin, "Peroxisomes (microbodies and related particles)," Physiological Reviews, vol. 46, no. 2, pp. 323–357, 1966.

[7] B. M. Elliott, N. J. F. Dodd, and C. R. Elcombe, "Increased hydroxyl radical production in liver peroxisomal fractions from rats treated with peroxisome proliferators," Carcinogenesis, vol. 7, no. 5, pp. 795–799, 1986.

[8] R. M. Zwacka, A. Reuter, E. Pfaff et al., "The glomerulosclerosis gene Mpv17 encodes a peroxiosomal protein producing reactive oxygen species," The EMBO Journal, vol. 13, no. 21, pp. 5129–5134, 1994.

[9] D. B. Stolz, R. Zamora, Y. Vodovoz et al., "Peroxisomal localization of inducible nitric oxide synthase in hepatocytes," Hepatology, vol. 36, no. 1, pp. 81–93, 2002.

[10] R. J. A. Wanders and H. R. Waterham, "Biochemistry of mammalian peroxisomes revisited," Annual Review of Biochemistry, vol. 75, pp. 295–332, 2006.

[11] J. M. Powers and H. W. Moser, "Peroxisomal disorders: genotype, phenotype, major neuropathologic lesions, and pathogenesis," Brain Pathology, vol. 8, no. 1, pp. 101–120, 1998.

[12] M. Baes and P. Aubourg, "Peroxisomes, myelination, and axonal integrity in the CNS," Neuroscientist, vol. 15, no. 4, pp. 367–379, 2009.

[13] A. Bottelbergs, S. Verheijden, P. P. Van Veldhoven, W. Just, R. Devos, and M. Baes, "Peroxisome deficiency but not the defect in ether lipid synthesis causes activation of the innate immune system and axonal loss in the central nervous system," Journal of Neuroinflammation, p. 61, 2012.

[14] D. Trompier, A. Vejux, A. Zarrouk et al., "Brain peroxisomes," Biochimie, 2013.

[15] A. D’Amico and E. Bertini, "Metabolic neuropathies and myopathies," Handbook of Clinical Neurology, vol. 113, pp. 1437–1455, 2013.

[16] C. R. Giordano and S. R. Terlecky, "Peroxisomes, cell senescence, and rates of aging," Biochimica et Biophysica Acta, vol. 1822, pp. 1358–1362, 2012.

[17] A. Cimini, S. Moreno, M. D’Amelio et al., "Early biochemical and morphological modifications in the brain of a transgenic mouse model of Alzheimer’s disease: a role for peroxisomes," Journal of Alzheimer’s Disease, vol. 18, no. 4, pp. 935–952, 2009.

[18] P. I. Wood, R. Mankidi, S. Ritchie et al., "Circulating plasmalogens and Alzheimer disease assessment scale-cognitive scores in Alzheimer patients," Journal of Psychiatry and Neuroscience, vol. 35, no. 1, pp. 59–62, 2010.

[19] M. Igarashi, K. Ma, F. Gao, H.-W. Kim, S. I. Rapoport, and J. S. Rao, "Disturbed choline plasmalogens and phospholipid fatty acid concentrations in Alzheimer’s disease prefrontal cortex," Journal of Alzheimer’s Disease, vol. 24, no. 3, pp. 507–517, 2011.

[20] J. Kou, G. G. Kovacs, R. Höftberger et al., "Peroxisomal alterations in Alzheimer’s disease," Acta Neuropathologica, vol. 122, no. 3, pp. 271–283, 2011.

[21] M. Fransen, M. Nordgren, B. Wang, and O. Apanasets, "Role of peroxisomes in ROS/RNS-metabolism: implications for human disease," Biochimica et Biophysica Acta, vol. 1822, pp. 1363–1373, 2012.

[22] R. Shi, Y. Zhang, Y. Shi, S. Shi, and L. Jiang, "Inhibition of peroxisomal beta-oxidation by thioredoxin increases the amount of VLCFAs and Abeta generation in the rat brain," Neuroscience Letters, vol. 528, pp. 6–10, 2012.

[23] R. L. Hunter and G. Bing, "Agonism of peroxisome proliferator receptor-gamma may have therapeutic potential for neuroinflammation and Parkinson’s disease," Current Neuropharmacology, vol. 5, no. 1, pp. 35–46, 2007.

[24] G. Landreth, Q. Jiang, S. Mandrekar, and M. Heneka, "PPAR agonists as therapeutics for the treatment of Alzheimer’s disease," Neurotherapeutics, vol. 5, no. 3, pp. 481–489, 2008.

[25] Y.-J. Lee, S. B. Han, S.-Y. Nam, K.-W. Oh, and J. T. Hong, "Inflammation and Alzheimer’s disease," Archives of Pharmacal Research, vol. 33, no. 10, pp. 1539–1556, 2010.

[26] M. V. Loureiro and J. H. Leo, "Targeting Alzheimer’s pathology through PPARγ signaling: modulation of microglial function," The Journal of Neuroscience, vol. 33, pp. 5083–5084, 2013.

[27] C. R. Swanson, V. Joers, V. Bondarenko et al., "The PPARγ agonist pioglitazone modulates inflammation and induces neuroprotection in parkinsonian monkeys," Journal of Neuroinflammation, vol. 8, article 91, 2011.

[28] V. Benedusi, F. Martorana, L. Brambilla, A. Maggi, and D. Rossi, "The peroxisome proliferator-activated receptor γ (PPARγ) controls natural protective mechanisms against lipid peroxidation in amyotrophic lateral sclerosis," The Journal of Biological Chemistry, vol. 287, no. 43, pp. 35899–35911, 2012.

[29] M. Napolitano, L. Costa, R. Palermo, A. Giovenco, A. Vacca, and A. Gulino, "Protective effect of pioglitazone, a PPARγ ligand, in a 3 nitropipionic acid model of Huntington’s disease," Brain Research Bulletin, vol. 85, no. 3–4, pp. 231–237, 2011.

[30] M. T. Heneka, G. E. Landreth, and M. Hüll, "Drug Insight: effects mediated by peroxisome proliferator-activated receptor-γ in CNS disorders," Nature Clinical Practice Neurology, vol. 3, no. 9, pp. 496–504, 2007.

[31] I. Issemam and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," Nature, vol. 347, no. 6294, pp. 645–650, 1990.

[32] G. D. Gurnun, F. E. Domann, S. A. Moore, and M. E. C. Robbins, "Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter," Molecular Endocrinology, vol. 16, no. 12, pp. 2793–2801, 2002.

[33] C. Li, R. Zhao, K. Gao et al., "Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer’s disease," Current Alzheimer Research, vol. 8, no. 1, pp. 67–80, 2011.

[34] M. Kipp, P. van der Valk, and S. Amor, "Pathology of multiple sclerosis," CNS & Neurological Disorders—Drug Targets, vol. 11, pp. 506–517, 2012.

[35] L. Brambilla, F. Martorana, and D. Rossi, "Astrocyte signaling and neurodegeneration: new insights into CNS disorders," Prion, vol. 7, pp. 28–36, 2013.

[36] K. D. McCarthy and J. De Vellis, "Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue," Journal of Cell Biology, vol. 85, no. 3, pp. 890–902, 1980.

[37] M. Ahmadian, J. M. Suh, N. Hah et al., "PPARγ signaling and metabolism: the good, the bad and the future," Nature Medicine, vol. 19, pp. 557–566, 2013.
C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

Y.-C. Chen, J.-S. Wu, H.-D. Tsai et al., "Peroxisome proliferator-activated receptor gamma (PPAR-γ) and neurodegenerative disorders," *Molecular Neurobiology*, vol. 46, pp. 114–124, 2012.

V. D. Antonenko, R. T. Sormunen, and J. K. Hiltunen, "The behavior of peroxisomes in vitro: mammalian peroxisomes are osmotically sensitive particles," *American Journal of Physiology*, vol. 287, no. 6, pp. C1623–C1635, 2004.

P. A. Loughran, D. B. Stolz, Y. Vodovotz, S. C. Watkins, R. L. Simmons, and T. R. Billiar, "Monomeric inducible nitric oxide synthase localizes to peroxisomes in hepatocytes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 39, pp. 13837–13842, 2005.

G. C. Brown and V. Borutaite, "There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells," *Mitochondrion*, vol. 12, no. 1, pp. 1–4, 2012.

C. Venkateshappa, G. Harish, A. Mahadevan, M. M. Srinivas Bharath, and S. K. Shankar, "Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer’s disease," *Neurochemical Research*, vol. 37, pp. 1601–1614, 2012.

A. Ghanizadeh, S. Akhondzadeh, M. Hormozi, A. Makarem, M. Abothorabi-Zarchi, and A. Firoozabadi, "Glutathione-related factors and oxidative stress in autism, a review," *Current Medicinal Chemistry*, vol. 19, pp. 4000–4005, 2012.

S. Mueller, A. Weber, R. Fritz et al., "Sensitive and real-time determination of H2O2 release from intact peroxisomes," *Biochemical Journal*, vol. 363, no. 3, pp. 483–491, 2002.

S. Cardaci, G. Filomeni, G. Rotilio, and M. R. Cirio, "p38MAPK/p53 signalling axis mediates neuronal apoptosis in response to tetrahydrobiopterin-induced oxidative stress and glucose uptake inhibition: implication for neurodegeneration," *Biochemical Journal*, vol. 430, no. 3, pp. 439–451, 2010.

S. Iwashita, M. Tsuchida, M. Tsukuda et al., "Multiple organelle-targeting signals in the N-terminal portion of peroxisomal membrane protein PMP70," *Journal of Biochemistry*, vol. 147, no. 4, pp. 581–590, 2010.

M. Kikuchi, N. Hatano, S. Yokota, N. Shimozawa, T. Imanaka, and H. Taniguchi, "Proteomic analysis of rat liver peroxisome. The behavior of peroxisomes in vitro: mammalian peroxisomes are osmotically sensitive particles," *American Journal of Physiology*, vol. 287, no. 6, pp. C1623–C1635, 2004.

G. C. Brown and V. Borutaite, "There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells," *Mitochondrion*, vol. 12, no. 1, pp. 1–4, 2012.

C. Venkateshappa, G. Harish, A. Mahadevan, M. M. Srinivas Bharath, and S. K. Shankar, "Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer’s disease," *Neurochemical Research*, vol. 37, pp. 1601–1614, 2012.

A. Ghanizadeh, S. Akhondzadeh, M. Hormozi, A. Makarem, M. Abothorabi-Zarchi, and A. Firoozabadi, "Glutathione-related factors and oxidative stress in autism, a review," *Current Medicinal Chemistry*, vol. 19, pp. 4000–4005, 2012.

S. Mueller, A. Weber, R. Fritz et al., "Sensitive and real-time determination of H2O2 release from intact peroxisomes," *Biochemical Journal*, vol. 363, no. 3, pp. 483–491, 2002.

S. Cardaci, G. Filomeni, G. Rotilio, and M. R. Cirio, "p38MAPK/p53 signalling axis mediates neuronal apoptosis in response to tetrahydrobiopterin-induced oxidative stress and glucose uptake inhibition: implication for neurodegeneration," *Biochemical Journal*, vol. 430, no. 3, pp. 439–451, 2010.

S. Iwashita, M. Tsuchida, M. Tsukuda et al., "Multiple organelle-targeting signals in the N-terminal portion of peroxisomal membrane protein PMP70," *Journal of Biochemistry*, vol. 147, no. 4, pp. 581–590, 2010.

M. Kikuchi, N. Hatano, S. Yokota, N. Shimozawa, T. Imanaka, and H. Taniguchi, "Proteomic analysis of rat liver peroxisome. The behavior of peroxisomes in vitro: mammalian peroxisomes are osmotically sensitive particles," *American Journal of Physiology*, vol. 287, no. 6, pp. C1623–C1635, 2004.
in vivo assessed with rat hippocampus lactography,” *Neuroscience Letters*, vol. 208, no. 1, pp. 69–72, 1996.

[70] J. Lotharius, J. Falsig, J. Van Beek et al., “Progressive degeneration of human mesencephalic neuron-derived cells triggered by dopamine-dependent oxidative stress is dependent on the mixed-lineage kinase pathway,” *Journal of Neuroscience*, vol. 25, no. 27, pp. 6329–6342, 2005.

[71] R. Resende, P. I. Moreira, T. Proença et al., “Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease,” *Free Radical Biology and Medicine*, vol. 44, no. 12, pp. 2051–2057, 2008.

[72] J.-H. Choi, D.-H. Kim, I.-J. Yun, J.-H. Chang, B.-G. Chun, and S.-H. Choi, “Zaprinast inhibits hydrogen peroxide-induced lysosomal destabilization and cell death in astrocytes,” *European Journal of Pharmacology*, vol. 571, no. 2-3, pp. 106–115, 2007.

[73] K. Takuma, A. Baba, and T. Matsuda, “Astrocyte apoptosis: implications for neuroprotection,” *Progress in Neurobiology*, vol. 72, no. 2, pp. 111–127, 2004.

[74] R. G. Giffard and R. A. Swanson, “Ischemia-induced programmed cell death in astrocytes,” *Glia*, vol. 50, no. 4, pp. 299–306, 2005.

[75] L. Di Cesare Mannelli, M. Zanardelli, P. Failli, and C. Ghelardini, “Oxaliplatin-induced oxidative stress in nervous system-derived cellular models: could it correlate with in vivo neuropa-thy?” *Free Radical Biology and Medicine*, vol. 61, pp. 143–150, 2013.

[76] Y. Hamdi, O. Masmoudi-Kouki, H. Kaddour et al., “Protective effect of the octadecaneuropeptide on hydrogen peroxide-induced oxidative stress and cell death in cultured rat astrocytes,” *Journal of Neurochemistry*, vol. 118, no. 3, pp. 416–428, 2011.

[77] C. M. Kassmann, C. Lappe-Siefke, M. Baes et al., “Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes,” *Nature Genetics*, vol. 39, no. 8, pp. 969–976, 2007.

[78] S. Ferdinandusse, A. W. M. Zomer, J. C. Komen et al., “Ataxia with loss of Purkinje cells in a mouse model for Refsum disease,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 46, pp. 17712–17717, 2008.

[79] A. Pujol, C. Hindelang, N. Callizot, U. Bartsch, M. Schachner, and J. L. Mandel, “Late onset neurological phenotype of the X-ALD gene inactivation in mice: a mouse model for adrenomyeloneuropathy,” *Human Molecular Genetics*, vol. 11, no. 5, pp. 499–505, 2002.

[80] M. Baes, P. Gressens, E. Baumgart et al., “A mouse model for Zellweger syndrome,” *Nature Genetics*, vol. 17, no. 1, pp. 49–57, 1997.

[81] L. Hulshagen, O. Krysko, A. Bottelbergs et al., “Absence of functional peroxisomes from mouse CNS causes dysmyelination and axon degeneration,” *Journal of Neuroscience*, vol. 28, no. 15, pp. 4015–4027, 2008.