PPAR-γ: therapeutic target for ischemic stroke

Juraj Culman, Yi Zhao, Peter Gohlke and Thomas Herdegen

Institute of Pharmacology, University Hospital of Schleswig-Holstein, Campus Kiel, Hospitalstrasse 4, 24105 Kiel, Germany

The peroxisome proliferator activated receptors (PPARs), which belong to the nuclear receptor superfamily, are key regulators of glucose and fat metabolism. The PPAR-γ isoform is involved in the regulation of cellular glucose uptake, protection against atherosclerosis and control of immune reactions. In addition, the activation of PPAR-γ effectively attenuates neurodegenerative and inflammatory processes in the brain. Here, we review a novel aspect of beneficial and clinically relevant PPAR-γ actions: neuroprotection against ischemic injury mediated by intracerebral PPAR-γ, which is expressed in neurons and microglia. Together with the recent observation that the PPAR-γ ligand pioglitazone reduces the incidence of stroke in patients with type 2 diabetes, this review supports the concept that activators of PPAR-γ are effective drugs against ischemic injury.

Introduction

Cerebral ischemia results from a transient or permanent local reduction of cerebral blood flow. With a mortality rate of ~30%, stroke is one of the leading causes of death and adult disability in industrialized countries. The poor prognosis of patients suffering from stroke results from the lack of effective therapies. Owing to the limited therapeutic window (up to three hours after the onset of symptoms), only a restricted number of hospitalized patients can profit from thrombolytic therapy [1]. Moreover, many neuroprotective agents that showed beneficial effects in preclinical studies either failed in clinical trial, displayed severe side-effects or worsened the outcome of stroke. The reasons for this apparent discrepancy between animal experiments and clinical application are multifactorial and are discussed elsewhere [2,3]. Thus, the dilemma of experimental success and practical failure encourages the search for new applications of already-approved drugs. In this context, recent data from animal experiments strongly indicate that ligands of the peroxisome proliferator activated receptor (PPAR)-γ confer neuroprotection and neurological improvement following cerebral ischemia. Importantly, PPAR-γ agonists are already approved for the treatment of type 2 diabetes as effective insulin sensitizers and, because these agents are commonly used in clinical practice, they are attractive neuroprotective agents.

In this review, we summarize the recent findings that PPAR-γ agonists protect against ischemic injury, with a particular focus on their intracerebral effects and their putative application against stroke.

PPARs – multifunctional agents against the metabolic syndrome and inflammation

PPARs belong to the nuclear receptor superfamily. They form heterodimers with the retinoid X receptor and bind to PPAR response elements in the promoter of their target genes. Three PPAR isoforms have been identified: PPAR-γ, PPAR-α and PPAR-δ (also called PPAR-β). The gene encoding PPAR-γ is located on chromosome 3 at position 3p25. Alternative splicing produces two isoforms: PPAR-γ1 and PPAR-γ2. PPAR-γ1 is expressed in most tissues, whereas PPAR-γ2 is restricted to adipose tissue [4,5].

In this review, we focus on the PPAR-γ protein, which increases insulin sensitivity and decreases insulin resistance in adipose tissue, skeletal muscle and liver. In the vascular system, PPAR-γ confers anti-atherosclerotic effects [6–9] (Figure 1). It further antagonizes the metabolic syndrome by downregulating peripheral inflammatory processes, including the suppression of proinflammatory cytokines and adhesion molecules [10,11]. Beyond these functions, recent data have shown that PPAR-γ acts as a regulator of CNS inflammation and is a powerful pharmacological target for counteracting neurodegeneration, as shown in animal models (e.g. of Parkinson’s and Alzheimer’s diseases [12,13]).

Multiple targets for PPAR-γ agonists in ischemic injury

Neurons that are localized in the ischemic core die rapidly after vascular occlusion because of ischemia-induced energy failure and anoxic depolarization. In the early ischemic phase, neuronal death is the consequence of excitotoxicity, which is characterized by a robust activation of glutamate receptors, calcium overload and a breakdown of ion homeostasis [14,15]. Moreover, the intraneuronal and extraneuronal production of reactive oxygen species (ROS) and free radicals directly affects neurons, and this enhanced production is stimulated by, for example, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) [16,17]. Delayed neuronal death is triggered by inflammatory reactions [18] that are initiated by the increased expression and/or release of cytokines such as tumor necrosis factor (TNF)-α and interleukin (Il)-1β, and adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM). These mediators promote the accumulation of leukocytes, macrophages and activated microglial cells in the ischemic area [19,20]. Infiltrating inflammatory cells express iNOS and produce large amounts of nitric oxide (NO), with the subsequent formation of peroxynitrite [21]. The activation of PPAR-γ can
antagonize these harmful effects, indicating a promising and neuroprotective role for PPAR-\(\gamma\) agonists in stroke.

**Activation of intracerebral PPAR-\(\gamma\) protects against cerebral ischemia**

PPAR-\(\gamma\) agonists demonstrably and efficiently protect against cerebral ischemia in rodents [12], and this protection includes a reduction in rates of apoptosis [22]. The relevance of PPAR-\(\gamma\) as an endogenous protective factor was also shown by the fact that treatment with a PPAR-\(\gamma\) antagonist increased infarct size [23]. Only recently, however, was it shown that this protection is brought about by the selective stimulation of intracerebral PPAR-\(\gamma\): the intracerebroventricular application of the PPAR-\(\gamma\) agonist pioglitazone was as effective as systemic application [24]. How do PPAR-\(\gamma\) and its agonists protect against ischemic brain injury and what changes can be achieved in addition to reduction of the infarct area?

**Activation of intracerebral PPAR-\(\gamma\)**

Ischemic injury enhances the expression of PPAR-\(\gamma\) mRNA and protein in neurons and microglia [22,23,25,26]; maximal levels are observed after 24 hours, and increased PPAR-\(\gamma\) protein levels can still be detected up to 14 days after ischemic injury [23]. However, increased PPAR-\(\gamma\) expression might not be functionally important because cerebral ischemia reduces the DNA binding of PPAR-\(\gamma\). Importantly, DNA binding is fully restored by intracerebral application of the PPAR-\(\gamma\) agonist 15-deoxy-\(\Delta^{12,14}\)-prostaglandin \(J_2\) (15-deoxy-PGJ\(_2\)) or by systemic treatment with rosiglitazone [23,25,27].

**Suppression of COX-2**

The activation of PPAR-\(\gamma\) in neurons raises the issue of the suppression of neurodegenerative target genes such as the one encoding COX-2, an enzyme involved in the production of ROS. Systemic and intracerebroventricular application of thiazolidinedione (TZD) PPAR-\(\gamma\) agonists reduced the expression of COX-2 in peri-infarct cortical zones following transient occlusion of the middle cerebral artery or common carotid arteries [26,28–30] (Table 1, Figure 2).

**Table 1. Neuroprotective effects of PPAR-\(\gamma\) ligands in brain ischemia**

<table>
<thead>
<tr>
<th>PPAR-(\gamma) ligand</th>
<th>Application (species)</th>
<th>Effects</th>
<th>Infarct size</th>
<th>Neurological outcome</th>
<th>Refs</th>
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</thead>
<tbody>
<tr>
<td>Pioglitazone</td>
<td>Oral (rat)</td>
<td>Upregulation of CuZn–superoxide dismutase</td>
<td>Reduced</td>
<td>Improved</td>
<td>[31]</td>
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<tr>
<td>Rosiglitazone and pioglitazone</td>
<td>i.p. (rat)</td>
<td>Reduced macrophage and microglia accumulation</td>
<td>Reduced</td>
<td>Improved</td>
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<td>Inhibition of COX-2 expression</td>
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<td></td>
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<td>Inhibition of IL-1(\beta) expression</td>
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<td>Inhibition of iNOS expression</td>
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<tr>
<td>Pioglitazone</td>
<td>i.c.v. (rat)</td>
<td>Reduced macrophage and microglia accumulation</td>
<td>Reduced</td>
<td>Improved</td>
<td>[24]</td>
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<tr>
<td>15-Deoxy-PGJ(_2)</td>
<td>i.c.v. (rat)</td>
<td>Increased PPAR-(\gamma) DNA binding</td>
<td>Reduced</td>
<td>ND</td>
<td>[25]</td>
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<td>Rosiglitazone</td>
<td>i.p. (rat)</td>
<td>Increased PPAR-(\gamma) DNA binding</td>
<td>Reduced</td>
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<td>[23]</td>
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<td>Increased lipoprotein lipase mRNA expression</td>
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<td>Rosiglitazone</td>
<td>i.p. (mouse)</td>
<td>Increased PPAR-(\gamma) DNA binding</td>
<td>Reduced</td>
<td>ND</td>
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<td>Attenuation of ICAM-1 expression</td>
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<td>Attenuation of MPO activity</td>
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<td>Attenuation of cytokine expression</td>
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<td>15-Deoxy-PGJ(_2) and 15-Deoxy-PGJ(_2) and rosiglitazone</td>
<td>i.c.v. (rat)</td>
<td>Increased PPAR-(\gamma) protein</td>
<td>Reduced</td>
<td>ND</td>
<td>[22]</td>
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<tr>
<td>Rosiglitazone and pioglitazone</td>
<td>i.v. (rat)</td>
<td>Inhibition of caspase-3 activation</td>
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<td>Reduced oxidative stress</td>
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<td>Prevention of glutathione depletion</td>
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<td>Reduction of COX-2 expression</td>
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<td>Prevention of p38 kinase activation</td>
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<td>Prevention of p42/p44 kinase activation</td>
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<td>L796449</td>
<td>i.p. (rat)</td>
<td>Inhibition of iNOS expression</td>
<td>Reduced</td>
<td>Improved</td>
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<td>Inhibition of MMP-9 expression</td>
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<td>Activation of HO-1 expression</td>
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<td>Increased binding of nuclear proteins to PPRE</td>
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<td>Pioglitazone</td>
<td>i.c.v. (rat)</td>
<td>Reduced COX-2 expression</td>
<td>Reduced</td>
<td>ND</td>
<td>[26]</td>
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<td>Reduced COX-1 expression</td>
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<td>Reduced TNF-(\alpha) expression</td>
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*Abbreviations: HO-1, heme oxygenase 1; i.c.v., intracerebroventricular; i.p., intraperitoneal; MPO, myeloperoxidase; ND, not determined; PPRE, peroxisome proliferator response element.
Antioxidative actions
In addition to downregulating COX-2 expression, PPAR-γ agonists affect the generation of ROS on various levels. For example, pioglitazone induces the expression of the antioxidant enzyme CuZn–superoxide dismutase, which scavenges free oxygen radicals in ischemic tissue [31]. The treatment of rats with either pioglitazone or rosiglitazone before occlusion of the common carotid artery decreased the production of ROS and nitrite, decreased lipid peroxidation and reversed the depleted stores of glutathione in the hippocampus [29]. Finally, TZD and non-TZD PPAR-γ agonists attenuate the expression of iNOS in inflammatory cells [30,32], which is considered to be an important source of the deleterious radical peroxynitrite.

Inhibition of inflammation
As shown recently, PPAR-γ and its agonists not only reduce immune reactions outside the nervous system but also reveal a powerful anti-inflammatory potential in ischemic brains. Activation of PPAR-γ attenuates the expression of ICAM-1, matrix metalloproteinase (MMP)-9 and various inflammatory cytokines in ischemic brain tissue [27,32]. The systemic treatment of rodents with rosiglitazone reduces the infiltration of microglia and macrophages into peri-infarct brain regions and downregulates the production of IL-1β [27,30]. Importantly, these anti-inflammatory effects, including the suppression of TNF-α, can also be achieved by exclusively stimulating intracerebral PPAR-γ with intracerebroventricular application of pioglitazone [24,26]. Finally, rosiglitazone and pioglitazone inhibit NF-κB signaling and the activation of p38 stress kinase [29].

Alleviation of neurological deficits
Reduction of the infarcted area per se, which is a primarily morphological feature, is not necessarily associated with improved neurological outcome, which is the major clinically relevant endpoint of each stroke treatment. Can PPAR-γ agonists ameliorate neurological functions? Indeed, TZD and non-TZD PPAR-γ agonists improve the level of recovery from ischemic stroke [30–32]; again, this improvement could be attributed to the stimulation of exclusively cerebral PPAR-γ, as demonstrated by the intracerebral application of pioglitazone [24]. The neuroprotective effects of PPAR-γ agonists are summarized in Figure 2 and Table 1.

Mode of application – intracerebral versus peripheral sites of action
PPAR-γ agonists improve the level of recovery from ischemic stroke regardless of the mode of application (e.g. systemic or intracerebroventricular). However, it remains to be elucidated to what extent the stimulation of peripheral, cerebrovascular, neuronal or microglial PPAR-γ contributes to neuroprotection. For example, long-term treatment with rosiglitazone promotes angiogenesis after focal cerebral ischemia [33]. The systemic application of pioglitazone in
humans prevents macrovascular events, including stroke [34], which strongly indicates that non-cerebral actions (e.g. the retardation of atherosclerosis) could contribute to the improvement of various brain pathologies. PPAR-γ agonists reduce oxidative stress and increase NO production in endothelial cells, which, in turn, can modulate cerebral blood flow and prevent TNF-α-mediated endothelial dysfunctions such as the expression of adhesion molecules and cytokines [35,36]. Overall, effective neuroprotection by PPAR-γ agonists is probably due to the combined targeting of peripheral (vascular or metabolic) and brain PPAR-γ receptors.

PPAR-γ and neuroprotection: in vitro observations

Several in vitro studies have provided detailed insights into the mechanism that underlies the neuroprotective actions of PPAR-γ agonists following ischemic injury. Consistent with neuronal PPAR-γ contributing to neuroprotection, thiadiazolidinone PPAR-γ agonists prevent the apoptosis of primary cortical neurons evoked by cell-free supernatant from lipopolysaccharide (LPS)-activated microglia [37]. We have demonstrated in primary cortical neurons that pioglitazone substantially reduces the induction of COX-2 and prevents neuronal death in response to oxidative injury, even in the absence of microglia [26]. Relevant to this, PPAR-γ agonists attenuate neuronal iNOS expression and protect cerebellar granule cells from cytokine-induced apoptotic death [38].

PPAR-γ agonists diminish excitotoxicity to a similar extent as do selective NMDA receptor antagonists. The PPAR-γ agonist troglitazone attenuates glutamate toxicity in retinal ganglion cells [39] and cerebellar granule cells [40]. Interestingly, PPAR-γ ligands are still neuroprotective when applied at time points following glutamate exposure at which glutamate antagonists fail to be protective [40]. The neuroprotective effect of PPAR-γ ligands is linked to increased PPAR-γ DNA binding and can be fully reversed by selective PPAR-γ antagonists [41].

The activation of macrophages and microglia following ischemia is associated with the secretion of neurotoxic molecules that promote neuronal death [14,42]. In cultured astrocytes and microglia, PPAR-γ ligands abolish LPS-induced iNOS expression and reduce the release of NO, TNF-α and IL-6 into the culture medium. In addition, COX-2 expression is reduced in neurons [37,43].

Studies in vivo and in vitro have demonstrated that neuroprotection by PPAR-γ activation following ischemia involves: (i) the inhibition of excitotoxicity; (ii) the reduced production of neurotoxic molecules in inflammatory cells; and (iii) the attenuation of harmful neuronal reactions to ischemic insults, such as enhanced COX-2 expression.

PPAR-γ and neuroplasticity

Successful stroke therapy should result in reduced infarct size and enhanced functional recovery. Interestingly, PPAR-γ ligands are similarly neurotrophic for rat motoneurons as is brain-derived neurotrophic factor (BDNF) [44] and, therefore, might support the structural recovery of surviving neurons. Furthermore, the nerve growth factor (NGF)-mediated differentiation of PC12 cells, which is an accepted model of neuronal differentiation, is associated with increased transcriptional activity of PPAR-γ [45]. The PPAR-γ ligand 15-deoxy-PGJ2 induces morphological differentiation in PC12 cells that is accompanied by enhanced neurite extension and expression of neurofilament proteins [46,47]. These data indicate that PPAR-γ ligands might support not only the structural but also the functional recovery of the brain following ischemic insults.

Post-ischemic versus pre-ischemic application of PPAR-γ agonists

What is the therapeutic time frame of PPAR-γ agonists for an effective treatment of stroke? Pioglitazone and rosiglitazone confer effective protection when applied between three and five days before experimental ischemia, and the therapeutic time frame extends to two hours after the onset of ischemia [27]. It is not yet clear whether the success of pre-treatment depends on the intracerebral accumulation of TZD, the upregulation of PPAR-γ expression or the slow alterations of effector proteins controlled by PPAR-γ, such as COX-2, iNOS, Bax and MMP (Table 1). PPAR-γ agonists are considered potent vasoprotective agents that contribute to the regression of harmful remodeling provoked by angiotensin II [48]; this long-lasting process might contribute to improved recirculation and enhanced blood supply in ischemic areas that are at risk not only after pre-ischemic but also after post-ischemic treatment. Therapeutic post-ischemic strategies should also address the upregulation of PPAR-γ expression. For example, the forkhead box class O (FOXO)1 transcription factor represses PPAR-γ promoter sites [4], and this repression could be antagonized by radical scavengers or antioxidative nutrients [49], resulting in a larger pool of PPAR-γ molecules.

Neurotoxicity following PPAR-γ activation

A striking biological feature is the ability of trophic or regenerative molecules to trigger harmful or even apoptotic cellular reactions. This bipartite action has been observed for the neurotrophin BDNF, the stress kinase JNK, the p75 trkA receptor and the death-inducing Fas ligand. Does this also hold true for PPAR-γ? At present, there is a small number of observations of the dose-dependent neurotoxic effects of the endogenous PPAR-γ ligand 15-deoxy-PGJ2 in cerebellar granule cells [50], primary cortical neurons [51] and spinal cord motor neurons [52]. The mechanism that underlies this neurotoxicity is unclear and some findings indicate that these harmful actions are probably not driven by PPAR-γ.

Clinical outlook: acute neuroprotection by PPAR-γ agonists

The reviewed data regarding neuroprotection against acute ischemic injury raise the issue of whether PPAR-γ agonists can be used in the treatment of stroke. So far, only TZD derivatives are of clinical importance. It is not clear whether the penetration of peripherally applied PPAR-γ agonists into the brain should be improved because penetration is substantially alleviated by the opening of the blood–brain barrier – a pathophysiological feature of cerebral ischemia. For example, pioglitazone attenuates the incidence of macrovascular events, including stroke,
in patients with type 2 diabetes [34]. This secondary prevention was observed after a long-term treatment (average observation of 34.5 months) with pioglitazone at doses ranging from 15 to 45 mg [34].

Which adverse side-effects must be taken into consideration? Full PPAR-γ agonists cause hemodilution, peripheral edema, an increase in body weight and deterioration of cardiomyopathies [34,53,54]. These, however, are long-term side effects and should not compromise the short-term application of PPAR-γ agonists after stroke, which might be sufficient for improved neurological outcome.

By contrast, PPAR-γ agonists have an excellent profile of extracerebral parameters that are relevant for the beneficial treatment of stroke. PPAR-γ agonists are the only neuroprotective strategy with a glucose-lowering component. This is of clinical interest because hyperglycemia worsens the neurological outcome after stroke [55]. TZD drugs weakly lower blood pressure compared with angiotensin-converting enzyme inhibitors or sartanes. This is of clinical relevance because a substantial reduction in blood pressure worsens the neurological outcome, which depends on effective reperfusion pressure [1,56]. Finally, PPAR-γ agonists provide further beneficial characteristics that are missing from other neuroprotective strategies, such as pronounced anti-inflammatory and anti-atherosclerotic actions.

Chemical names

FK614: 3-(2,4-dichlorobenzyl)-2-methyl-N-(pentylsulfonyl)-3-H-benzimidazole-5-carboxamid
L796449: 3-chloro-4-[3-(3-phenyl-7-propylbenzofuran-6-oxyl)-propylthio] phenyl acetic acid

Concluding remarks

Although TZD has received the most attention, non-TZD PPAR-γ agonists such as L796449 (see Chemical names) also enhance neuroprotection by activating PPAR-γ-dependent and PPAR-γ-independent pathways [32]. Other promising agonists include selective non-TZD PPAR-γ modulators such as FK614 (in Phase II clinical trials) and dual PPAR-α–PPAR-γ agonists [7]. The translation of preclinical findings into the appropriate study design [3] and the correct interpretation of data, as controversially discussed for the study carried out with the α-phenyl-N-tert-butyl nitroline derivative NXY059 [57], are a challenge for the future, and more information must be generated before PPAR-γ agonists can be tested for the treatment of acute stroke. It is the combined targeting of insulin resistance, hyperlipidemia, atherosclerosis, peripheral inflammation, neuronal apoptosis and cerebral inflammation, together with approved everyday application in diabetic patients, that renders PPAR-γ agonists the most promising and unique neuroprotective drugs. A first case-matched controlled study reporting improved functional recovery in stroke patients with type 2 diabetes [58] yields a promising outlook.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 415, project A12).

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