PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases

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Abstract

PPARs (peroxisome-proliferator-activated receptors) are ligand-activated transcriptional factor receptors belonging to the so-called nuclear receptor family. The three isoforms of PPAR (α, β/δ and γ) are involved in regulation of lipid or glucose metabolism. Beyond metabolic effects, PPARα and PPARγ activation also induces anti-inflammatory and antioxidant effects in different organs. These pleiotropic effects explain why PPARα or PPARγ activation has been tested as a neuroprotective agent in cerebral ischaemia. Fibrates and other non-fibrate PPARα activators as well as thiazolidinediones and other non-thiazolidinedione PPARγ agonists have been demonstrated to induce both preventive and acute neuroprotection. This neuroprotective effect involves both cerebral and vascular mechanisms. PPAR activation induces a decrease in neuronal death by prevention of oxidative or inflammatory mechanisms implicated in cerebral injury. PPARα activation induces also a vascular protection as demonstrated by prevention of post-ischaemic endothelial dysfunction. These vascular effects result from a decrease in oxidative stress and prevention of adhesion proteins, such as vascular cell adhesion molecule 1 or intercellular cell-adhesion molecule 1. Moreover, PPAR activation might be able to induce neurorepair and endothelium regeneration. Beyond neuroprotection in cerebral ischaemia, PPARs are also pertinent pharmacological targets to induce neuroprotection in chronic neurodegenerative diseases.

The treatment of ischaemic stroke is limited to the prevention of cerebrovascular risk factors and to the modulation of the coagulation cascade during the acute phase. During the last two decades, many drugs have been developed to induce neuroprotection during stroke [1,2]. Nevertheless, none of them has been successful at the clinical step of their development, while recent results give hope of a new antioxidant drug [3]. One of the explanations for this failure is that the developed drugs are able to modulate only one molecular pathway, while several pathways are involved spatially and temporally in the pathophysiology of stroke. One of the keys to success in inducing neuroprotection in stroke could be to modulate simultaneously many pathophysiological pathways with a combination of several drugs or, better, with only one pharmacological agent with pleiotropic effect. Such a pleiotropic effect can be induced by drugs acting on transcription factor receptors (so-called nuclear receptors), because this subtype of receptor is able to regulate several genes simultaneously. Among nuclear receptors, PPAR (peroxisome-proliferator-activated receptors) have been demonstrated to induce pleiotropic effects in different organs (vessels, heart and kidney) when activated by agonists. Some results support the idea that these pleiotropic effects could be useful to induce a neuroprotective effect in stroke [4,5].

PPARs: function and pharmacology

PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily [6] (Figure 1). Three isoforms of PPARs (α, β/δ and γ) have been identified, displaying distinct physiological and pharmacological functions depending on their target genes and their tissue distribution [7,8]. Indeed, the activation of PPARα, by both natural ligands such as fatty acids and eicosanoid derivatives or synthetic ligands (lipid-lowering fibrates), regulates lipid and lipoprotein metabolism [4] (Figure 2). Activation of PPARγ by prostaglandins or by synthetic ligands such as antidiabetic thiazolidinediones regulates glucose metabolism by modulation of insulin-sensitivity [4]. Non-steroidal anti-inflammatory drugs are also weak agonists of PPARγ and PPARα. PPARβ/δ is one of the most widely expressed members of the PPAR family. Until recently, the function of PPARβ/δ remained elusive, but recent results have shown that PPARβ/δ plays also a key role in lipid metabolism, as it regulates serum lipid profiles and fatty acid oxidation in muscle and adipose tissue. Synthetic ligands of PPAR β/δ are at the moment in preclinical phases of development [9].

Key words: cerebral ischaemia, neurodegenerative disease, neuroprotection, nuclear receptor, peroxisome-proliferator-activated receptor (PPAR), thiazolidinediones.

Abbreviations used: Aβ, amyloid β-peptide; CNS, central nervous system; COX, cyclooxygenase; EAE, encephalomyelitis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF-κB, nuclear factor κB; PPAR, peroxisome-proliferator-activated receptor.

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PPARs as regulators of inflammation and oxidative stress

Beyond effects on metabolic pathways, PPAR are also able to regulate inflammatory pathway by transgression of transcription factors [NF-κB (nuclear factor-κB)] or to regulate the oxidative pathway [5,6] (Figure 3). PPARα activation induces expression and activation of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. On the inflammatory pathway, PPARα activation prevents synthesis and release of cytokines (interleukin-6 and tumour necrosis factor α) or induction of some inflammatory mediators such as COX-2 (cyclo-oxygenase 2) and adhesion proteins. PPARγ activation also reduces the expression of inducible nitric oxide synthase or COX-2 as well as the production of pro-inflammatory cytokines. These effects on inflammation explain why activation of PPAR by synthetic ligands reduces inflammation in different tissues and in different animal models of inflammatory diseases (vascular inflammation of atherosclerosis, inflammatory bowel disease, arthritis etc.) [4,5].

PPAR in the brain: a potential target against neuronal death

Previously, it has been supposed that PPAR activation could also be effective in the regulation of neuronal death in ischaemic, neurodegenerative or inflammatory cerebral diseases. Firstly, PPARs have been described in brain and in spinal cord [10,11]. Beyond expression in cerebral or spinal blood vessels, PPARs are also expressed in neurons and in astrocytes, whereas oligodendrocytes exclusively show PPARβ/δ expression (Figure 4). The extent of this expression depends on the isoform of PPAR involved. PPARβ/δ has been found in numerous brain areas, while PPARα and PPARγ have been localized to more restricted brains areas [11]. Secondly, whatever the aetiology, neuronal death is induced by inflammatory and oxidative processes with a link between the two phenomena [12]. Inflammation and oxidative stress induce both necrotic and apoptotic neuronal death. The transcription factor NF-κB plays a key role in regulation of inflammation and oxidative stress leading to neuronal death, explaining why PPARs have been considered as possible targets for neuroprotection [13]. In vitro studies have demonstrated that PPARγ agonists modulate inflammatory responses to bacterial endotoxin in brain and also prevent endotoxin-induced neuronal death [14]. PPARγ agonists are able to prevent neuronal death resulting from NMDA (N-methyl-D-aspartate) excitotoxicity induced in brain in vitro or in vivo [15]. PPARα and PPARγ are able to inhibit macrophage and microglial activation that...
contribute to many degenerative, ischaemic or inflammatory processes leading to neuronal death [16]. Troglitazone and ciglitazone inhibit both post-glutamate- and low-potassium-induced neurotoxicity in cerebellar granule neurons [17]. PPARs are also able to inhibit the entry of inflammatory cells into the CNS (central nervous system) from the periphery by inhibition of chemokines, adhesion molecules and metalloproteinases [16].

**Figure 3** | Inflammation regulation is a common effect of PPAR and results from modulation of NF-κB

- PPAR-responsive element; RXR, retinoid X receptor.

**Figure 4** | Expression of PPAR in the three cellular types of the neuro-glio-vascular unit

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**PPAR and cerebral ischaemia**

**PPAR-induced neuroprotection in cerebral ischaemia**

Because fibrates, used as lipid-lowering agents, contribute to secondary prevention of stroke, it has been supposed that these PPARα activators could also preventively protect the brain against noxious biological reactions induced by cerebral
ischaemia, such as oxidative stress and inflammation. It has been demonstrated that a 14-day preventive treatment with fenofibrate reduced susceptibility to stroke in apolipoprotein E-deficient mice as well as decreased cerebral infarct volume in wild-type mice [18]. In another study, it was confirmed that two different PPARγ agonists, fenofibrate and Wy-14643, provided similar brain protection when administered respectively 3 or 7 days before induction of cerebral ischaemia [19]. More recently, it has been demonstrated that PPARγ agonists could also induce an acute neuroprotection when administered just before cerebral ischaemia or during the reperfusion period [20,21].

Administration of the PPARγ agonists troglitazone or pioglitazone 24 or 72 h before and at the time of cerebral infarction dramatically reduced infarct volume and improved neurological function following transient middle cerebral artery occlusion in rats [22,23]. This effect is exerted in a dose-dependent manner. This neuroprotection has been reproduced by an intracerebroventricular administration of pioglitazone, proving that it is the activation of intracerebral PPARγ that confers neuroprotection and neurological improvement following ischaemic injury [24]. Moreover, a non-thiazolidinedione PPARγ agonist (L-796449) also had a neuroprotective effect in experimental stroke and was also found to activate 15-deoxy-Δ12,14-prostaglandin J2 by adeno viral transfer of COX-2 [25,26]. In a first study, PPARγ agonists had no effect in a permanent model of cerebral ischaemia, suggesting that mechanisms of action could take place during reperfusion [23], while recent results give the opposite effect [25].

Mechanisms of PPAR-induced neuroprotection

Cerebral mechanisms

The neuroprotection observed after treatment with PPAR agonists is related to several mechanisms including both oxidative stress modulation and anti-inflammatory effect. PPARα agonist-induced neuroprotective effect is associated with a decrease in cerebral oxidative stress depending on the increase in activity of numerous antioxidant enzymes, in particular Cu/Zn superoxide dismutase and glutathione peroxidase [18]. This modulation of antioxidant enzymes is responsible for a decrease in ischaemia-induced reactive oxygen species production and lipid peroxidation [21,27]. This effect on oxidative stress could be related to a direct effect on antioxidant enzymes expression, because PPREs (PPAR-response elements) have been found in the gene of Cu/Zn superoxide dismutase [5].

The neuroprotective effects of PPAR agonists are also related to inhibition of ischaemia-induced inflammatory markers (interleukin-1β, COX-2 and inducible nitric oxide synthase) [21,27]. The different PPAR isoforms do not modulate the inflammatory pathways involved in neuroprotection in a similar manner. For instance, ischaemia-induced COX-2 overexpression is prevented by PPARγ agonists but not by PPARα agonists [21,22,27]. There is a link between PPAR-induced modulation of oxidative stress and inflammation, since prevention of COX-2 induction results from oxidative stress inhibition [28]. The cellular target of these anti-inflammatory effects is probably microglial cells, since PPARγ agonists, such pioglitazone, are able to decrease microglial activation when administered intracerebrally [24,29].

The key target of this anti-inflammatory effect is NF-κB, which plays a crucial role in neuronal death [30]. PPARγ and PPARα activation is responsible for inhibition of the NF-κB p65 monomer as well as induction of IκBα (inhibitory κB) [25,31]. The role of suppression of activation of p38 mitogen-activated protein kinase has also been demonstrated recently [21,27].

Beyond this direct effect on ischaemia-induced deleterious pathways explaining neuroprotection, the challenge will be to demonstrate that a part of the neurological improvement induced by PPAR activators could be the result of neurorepair, since PPARγs are also involved in the regulation of neural stem cell proliferation and differentiation [32].

Vascular mechanisms

Because PPARs are mainly expressed in cerebral vascular wall, in particular in endothelium, it has been supposed that vascular mechanisms could be involved in neuroprotection. Thus preventive neuroprotection by PPARα is associated with an improvement in middle cerebral artery sensitivity to endothelium-dependent relaxation unrelated to an increase in endothelial nitric oxide synthase expression [18]. More recently, it has been demonstrated that preventive or acute PPARα agonist-induced neuroprotection paralleled the prevention of ischaemia-induced endothelial dysfunction [20]. This vascular effect could be related to: (i) the prevention of ischaemia-induced vascular expression of adhesion molecules; (ii) the antioxidant effect of PPAR activation; and (iii) the inhibition of ischaemia-induced metalloprotease expression [18,25]. In addition, PPAR could also be involved in endothelial regeneration as has been demonstrated in other arterial areas [33].

PPAR and neuroprotection: beyond cerebral ischaemia

Other acute cerebral injuries such as traumatic brain injury or chronic neurological diseases such as neurodegenerative diseases or multiple sclerosis also need pleiotropic neuroprotective drugs, explaining why PPAR activators have also been tested in experimental models mimicking these different disorders.

Traumatic brain and spinal cord injury

Because many mechanisms that are involved in cerebral ischaemia are also involved in traumatic nervous tissue injury, the effect of PPARα activation has been tested in models of traumatic spinal cord and brain injury, and a neuroprotective effect has been observed with some similar mechanisms to those in cerebral ischaemia [34,35].

Alzheimer’s disease

PPARγ and PPARα agonists have been tested in models of Alzheimer’s disease. The classical histopathological hallmarks
of Alzheimer's disease include extracellular Aβ (amyloid β-peptide) deposition in neuritic plaques and intracellular deposits of hyperphosphorylated tau protein, causing formation of neurofilibrillary tangles and finally neuronal death, responsible for progressive memory loss and decline of cognitive functions. While it has been demonstrated that a PPARα agonist inhibited Aβ-stimulated expression of tumour necrosis factor α and interleukin-6 reporter genes in a dose-dependent manner, but failed to inhibit Aβ-stimulated elaboration of neurotoxic factors [36], some recent experimental data suggest that fenofibrate could raise Aβ-(1–42) production [37], suggesting that PPARα remains a controversial target in Alzheimer's disease. PPARγ agonists were also shown to inhibit the β-amyloid-stimulated expression of inflammatory cytokines and COX-2 [38]. In addition to inhibition of Aβ-induced inflammation, PPARγ could also induce clearance of the β-amyloid peptide [39]. In addition to in vitro data, recent in vivo data also indicate a beneficial effect of PPARγ activation, since an acute 7-day oral treatment with the PPARγ agonist pioglitazone resulted in a reduction in glial activation as well as a reduction in the number of Aβ-positive plaque areas in the hippocampus and cortex of a murine transgenic model of the amyloid pathology of Alzheimer's disease [40].

Parkinson's disease

PPAR agonists have also been assessed in a model of Parkinson's disease. Parkinson's disease is characterized by a progressive loss of dopaminergic neurons in the substantia nigra, which is experimentally mimicked by systemic administration of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Oral administration of the PPARγ agonist pioglitazone attenuated the MPTP-induced glial activation and prevented dopaminergic cell loss in the substantia nigra pars compacta. Pioglitazone also prevented MPTP-induced expression of inducible nitric oxide synthase [41]. This protective effect of pioglitazone is also associated with an increase in inhibitory protein-κBα expression and to inhibition of translocation of the NF-κB subunit p65 to the nucleus in dopaminergic neurons, glial cells and astrocytes [42]. Preliminary results demonstrate that PPARα activation prevents death of dopaminergic neurons of substantia nigra pars compacta in the MPTP model of Parkinson's disease [43].

Multiple sclerosis

Microglial activation and inflammation are the key to the pathophysiology of multiple sclerosis, explaining why PPAR agonists have been tested in this disease, in particular in the model of experimental autoimmune EAE (encephalomyelitis), which is characterized by CNS inflammation and demyelination, together with remittent paralysis [16]. Oral administration of gemfibrozil and fenofibrate, two PPARα agonists, also induces clinical signs of EAE by mechanisms involving secretion of interferon-γ and interleukin-4 [44]. Oral administration of the PPARγ agonist pioglitazone reduces the motor symptoms’ severity in monophasic EAE, without delaying the disease onset. In a relapsing model of EAE, pioglitazone reduces the severity of relapses and overall mortality without affecting the onset and severity of the initial disease attack [45]. The mechanisms of action of PPAR agonists in EAE are complex, involving regulation of the inflammatory pathway and also modulation of the maturation and differentiation of oligodendrocytes [16].

Conclusion

The hypothesis that the pleiotropic effects of PPAR agonist could decrease neuronal death is supported by much experimental data showing that PPAR agonists exert neuroprotective effects in models of cerebral ischaemia, neurodegenerative diseases and multiple sclerosis, with some clinical data confirming these experimental results. These results have been essentially obtained with PPARγ and PPARα activators, while the PPARβ/δ pathway remains largely unexplored despite interest in the target. Development of new and more potent PPAR activators as well as combined action of the different isoforms of PPAR are also future prospects in terms of neuroprotection and also in terms of neurorepair.

References


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