A REVIEW: ANTI-ARTHRITIC POTENCIES OF PPAR-α AND PPAR-γ AGONIST IN RHEUMATOID ARTHRITIS

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ABSTRACT
Rheumatoid arthritis is characterized by synovial hyperplasia, inflammatory infiltration, cartilage destruction and juxta-articular as well as generalized bone demineralization. Peroxisome proliferator-activated receptors (PPARs) were discovered over a decade ago, and were classified as orphan members of the nuclear receptor super family. To date, three PPAR subtypes have been discovered and characterized (PPAR α, β/δ, γ). Different PPAR subtypes have been shown to play crucial roles in important diseases and conditions such as obesity, diabetes, atherosclerosis, cancer, and fertility. Among the most studied roles of PPARs is their involvement in inflammatory processes. Numerous studies have revealed that agonists of PPAR α and PPAR γ exert anti-inflammatory effects both in vitro and in vivo. Using the carrageenan-induced paw oedema model of inflammation, a recent study in our laboratories showed that these agonists hinder the initiation phase, but not the late phase of the inflammatory process. Furthermore, in the same experimental model, we recently also observed that activation of PPAR δ exerted an anti-inflammatory effect. Despite the fact that exclusive dependence of these effects on PPARs has been questioned, the bulk of evidence suggests that all three PPAR subtypes, PPAR α, δ, γ, play a significant role in controlling inflammatory responses. Whether these subtypes act via a common mechanism or are independent of each other remains to be elucidated. However, due to the intensity of research efforts in this area, it is anticipated that these efforts will result in the development of PPAR ligands as therapeutic agents for the treatment of inflammatory diseases.

KEYWORDS: Adjuvant arthritis, PPARs, bone, Rheumatoid arthritis, PPARγ, PPARα.

INTRODUCTION
Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor super family, which behave as ligand-activated transcription factors in response to endogenous fatty acids and eicosanoids or iso type selective synthetic compounds.\footnote{1}

Peroxisome proliferator-activated receptors (PPARs) belong to the super family of steroid-thyroid-retinoid nuclear receptors. PPARs are transcription factors activated by specific ligands and play an important role during cell signalling. Intensive study of PPARs during recent years has revealed their importance in both normal physiology and in the pathology of various tissues. They participate in the regulation of lipid metabolism, inflammation and the development of...
atherosclerosis or diabetes. They also play a role in the regulation of growth and differentiation of cancer. It has been suggested that PPAR ligands may have potent anticancer effects and therefore may serve as potential anticancer drugs. In cell biology, peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor isoforms that exist across biology. Originally identified in xenopus. Frogs as receptors that induce the proliferation of peroxisome in cells, they are intimately connected to the cellular metabolism (carbohydrate, lipid and protein) and cell differentiation. They are transcription factor.[2]

**Nomenclature:**

Three types of PPARs have been identified: alpha, gamma and delta (beta).

- **α (alpha)** - expressed in kidney, heart, muscle, adipose tissue, and others.
- **γ (gamma)** - although transcribed by the same gene, this PPAR exists in three forms:
  - γ1 - expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas and spleen.
  - γ2 - expressed mainly in adipose tissue (30 amino acids longer)
  - γ3 - expressed in macrophage, large intestine, white adipose tissue.
  - γ4 - expressed in endothelial cells.
- **δ (delta)** - expressed in many tissues but markedly in brain, adipose tissue and skin.

Among the three characterized isotypes, PPAR-α is expressed mainly in tissues contributing actively to the catabolism of fatty acids and contributes to the control of inflammation. PPAR-γ is highly expressed in adipose tissue, where it has a pivotal role in adipocyte differentiation and lipid storage and is also a prominent player in inflammation control [1, 2]. All PPARs are expressed in both human and rodent osteoblasts and osteoclasts, supporting a role for the PPARs in the regulation of bone metabolism, although the significance of PPAR-α and its agonists in bone metabolism remains poorly elucidated. More is known about the role of PPAR-γ which agonists promote adipocyte differentiation preferentially over osteoblast differentiation [3].

Because of their metabolic actions, PPAR-α and PPAR-γ have become major drug targets [4]. Fibrates which well-known PPAR-α agonists, and are widely used for treatment of hypercholesterolemia and hypertriglyceridemia. Thiazolidinediones are synthetic PPAR-γ agonists previously used for treatment of type 2 diabetes mellitus. Experimental model of Adjuvant-Induced Arthritis (AIA) in rats reproduces major features upcoming in rheumatoid arthritis (RA). Its relevance to the pathogenesis of RA is further supported by the demonstration that pro-inflammatory cytokines are highly expressed in the developing arthritic process and by the reproduction of most bone changes found in RA[5], including inflammatory bone loss, which
has been linked to an increased risk of fracture. Others demonstrated previously that PPAR agonists reduced the severity of experimental polyarthritis and the overall inflammatory-induced bone loss. In the present study, we compare the effect of a PPAR-\(\alpha\) and a PPAR-\(\gamma\) agonist on arthritis development and secondary bone loss.\[^6\]

Table 1. Main Ligands, Tissue Distribution and Functions of the Two Peroxisome Proliferator-Activated Receptor (PPAR) Isotypes:\[^7\]

<table>
<thead>
<tr>
<th>PPAR-(\alpha)</th>
<th>Ligands</th>
<th>Tissue distribution</th>
<th>Functions</th>
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<tbody>
<tr>
<td>Fibrates (hypolipidaemic drugs)</td>
<td>Liver</td>
<td>Peroxisome proliferation (only rodents)</td>
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<td>Lipoxigenase metabolites: leukotriene B4 and 8(S)-hydroxy-eicosatetraenoic acid</td>
<td>Brown adipose tissue</td>
<td>Lipid catabolism</td>
<td></td>
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<td></td>
<td>Skeletal muscle, heart, kidney, and enterocytes</td>
<td>Control of inflammation</td>
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<td>Keratinocyte differentiation and proliferation Skin wound healing</td>
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<tr>
<th>PPAR-(\gamma)</th>
<th>Ligands</th>
<th>Tissue distribution</th>
<th>Functions</th>
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</thead>
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<tr>
<td>Thiazolidinediones: rosiglitazone, pioglitazone 15-deoxy- [12,14]-prostaglandin J2 9- and 13-HODE (hydroxyoctadecadienoic acid)</td>
<td>Adipose tissue</td>
<td>Lipid anabolism (storage)</td>
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<td></td>
<td>Macrophages Colon, spleen, retina, haematopoietic cells</td>
<td>Adipocyte differentiation</td>
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<td>Control of inflammation</td>
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<td>Macrophage maturation</td>
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<td>Embryo implantation</td>
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<td>Molecular target of the antidiabetic glitazones</td>
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MECHANISM OF ACTION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS: [8]

PPARs function as heterodimer in association with co-activator complex that binds to DNA sequence termed peroxisome proliferators response elements (PPREs) present in promoter of target genes which leads to transactivation and transrepression of various genes.[1] In the absence of the ligands, these heterodimers are associated with co-repressor complex which block gene transcription. Some of the agonists of various PPARs receptors are given in Balakumar P.2007.[5] Like PPARs, RXR exists as three distinct isoforms: RXR-α, β, and γ, all of which are activated by the endogenous agonist 9-cis retinoic acid.[6] No specific roles have yet been elaborated for these different isoforms within the PPAR: RXR complex. However, synthetic RXR agonists (rexiniids) can activate the complex and thereby obtain antidiabetic outcomes similar to those seen with PPAR agonists in mouse models of type 2 diabetes.[7] The LBD facilitates the heterodimerization of PPARs with RXR and the resultant heterodimer subsequently binds to PPRE with the recruitment of cofactors.[1]

IMPORTANCE OF PPAR α and PPAR γ [9,10,11]

- PPARs play an important role in many cellular functions including lipid metabolism, cell proliferation, differentiation, adipogenesis and inflammatory signaling. PPARs have been found to interact with a number of endogenous lipids and drugs for the treatment of human metabolic diseases.
- PPARs possess a highly conserved DNA binding domain (region C) and a diverse ligand-independent activation domain (region A/B), which can confer constitutive activity on the receptor.
- In the case of PPARα, insulin enhances transcriptional stimulation by phosphorylating the MAP kinase sites Ser 12 and Ser 21, whereas MAP kinase-mediated phosphorylation of Ser 112 of mouse PPAR.2 lowers transcriptional activity.
- PPAR-gamma also influences the prenatal survival of dizygotic (no identical) twins. Twins with the same type of PPAR-gamma may develop at the same pace. However, when the twins are carrying different versions of PPAR-gamma, one twin may develop at the expense of the other.
- PPAR α is expressed in tissues exhibiting high rates of β-oxidation such as liver, kidney, heart and muscle. In liver, PPARa regulates lipid metabolism and in rodents, But not in man, PPARa activation induces hepatomegaly and proliferation of liverPeroxisomes.
PPAR. Is highly expressed in adipose tissue and is a key transcription factor involved in the terminal differentiation of white and brown adipose tissue.

- PPARs regulate gene expression by complexing with a heterodimeric partner (retinoid X receptors) and subsequent binding to specific response elements (PPREs) in the promoter regions of target genes. With the involvement of PPARs in many diverse metabolic pathways, there is great clinical interest in the potential utility of PPAR ligands for the treatment of cancer, inflammation, psoriasis, atherosclerosis, dyslipidaemia, neurological disorders, obesity and diabetes.

**ROLE OF PPARs IN INFLAMMATION:**

Both PPAR α and PPAR γ receptor subtypes have been reported to regulate inflammatory responses, both in vivo and in vitro [12, 13]. However, the extent of this regulation, and indeed its direction, are controversial. No published reports are readily available on the involvement of PPAR δ in inflammation control. Potential modulation of inflammatory responses by PPAR δ agonists has only been recently investigated in laboratories.

**PPAR α:**

**Reduction of Leukotriene production by PPAR α agonists:**

The first indication of a role by PPAR in modulating inflammation was evidenced by the demonstration that Leukotriene B4 (LTB4), a potent chemotactic inflammatory eicosanoid [14], binds to PPAR α and induces transcription of genes of the ω- and β-oxidation pathways that can catabolize LTB4 itself [9]. PPAR α null mice showed a prolonged inflammatory response when challenged with LTB4 or its precursor, arachidonic acid, possibly due to the absence of stimulation of the catabolic pathways, hence, the increased duration of the inflammation [9]. Furthermore, dietary n-3 fatty acids and clofibrate, which also bind to PPAR α, have been reported to accelerate catabolism of LTB4 in granulocytes and macrophages [15, 16]. It is postulated that activation of PPAR α by non-steroidal anti-inflammatory agents contributes to the anti-inflammatory, antipyretic, and analgesic properties of these drugs through stimulation of oxidative pathways involved in the catabolism of eicosanoids [7].
Regulation of prostaglandins production by PPAR α:
Inhibition of the synthesis of pro-inflammatory molecules such as IL-6 and prostaglandins also appears to participate in PPAR α mediated control of inflammation, via a decreased activity of NF-κB [17]. Conversely, dietary treatment with PPAR α agonist’s increased lip polysaccharide-
induced plasma TNF α Levels, an effect that was significantly diminished in PPAR α deficient mice [18]. These latter results suggest a pro-inflammatory role of PPAR α.

Figure 2. Site-specific inhibition of carrageenan induced paw inflammation by PPAR agonists. The hatched bar indicates the site at which PPAR agonists interfere with the inflammatory process.

**Degradation of LTB4 by PPAR α:**
The postulate that PPAR α receptor activation enhances the degradation of lipid-derived pro-inflammatory mediators, such as LTB4 [9], is not supported by recent findings in our laboratories, using the carrageenan-induced rat paw edema model of inflammation [19]. First, we observed that the PPAR α agonist perfluorooctanoic acid (PFOA) produced robust anti-edema effects when administered minutes before carrageenan [19], yet de novo synthesis of enzymes requires a much longer time period, and is therefore expected to take hours following the administration of PPAR agonists. Second, when sufficient time for de novo enzyme synthesis was allowed by administering PFOA 12 or 24 hours before carrageenan, we did not observe an enhanced anti-edema effect compared to PFOA administered only 30 minutes before carrageenan [19]. Consequently, we speculate that PFOA inhibits the levels of pro-inflammatory mediators released during the induction of inflammation (Figures1, 2)

**Inhibition of transforming growth factor (TGF)-β by PPAR α:**
The transforming growth factor (TGF)-β stimulated production of proteoglycans and collagen in chondrocytes. PPAR α ligands inhibited the stimulatory effect of TGF-β1 on tissue inhibitor of matrix metalloproteinase 1 gene expression and protein synthesis. Results in reduced the inflammation and destruction of cartilage.
PPAR γ:

Role of PPAR γ in immune cells:

In vitro studies with cell lines and isolated primary cells proved to be essential in starting to understand the role of PPAR γ in immune responses and in evaluating the therapeutic potential of its agonists. These studies helped us to gain insights into the specific contributions of the different cell types in complex processes such as inflammation and inflammatory diseases. In connection with a certain cell type the basic questions are: whether the cell type expresses PPAR γ (and if yes, which isoform) in humans and in model animals and what kind of stimuli can regulate its expression level; how the presence of the receptor and its activation or inhibition alter inflammatory responses and which genes and gene networks are directly or indirectly regulated by the receptor. Estimating the availability and/or production of endogenous ligands under physiological conditions in a given cell type is also highly relevant, but quite a challenging issue and rarely addressed.

Figure 3 Effects of PPAR γ ligands on various immune cell types.
Reduction of nitric oxide (NO) production by PPAR γ agonists:

NO acts not only as a signal molecule mediating various physiological functions, but it also play an important role in inflammatory processes [21, 22]. Injection of LPS and IFN-γ into rat cerebellum induced the expression of iNOS, which produces NO, in cerebellar granule cells and caused subsequent cell death [23]. In this model, PPAR γ agonists reduced iNOS expression and cell death, whereas a selective COX-2 inhibitor had no effect [23]. Furthermore, mesangial cell production of NO was inhibited by PPARγ agonists [24]. These findings suggest that PPAR γ regulates the activity of iNOS and activation of this PPAR subtype controls inflammation by diminishing NO production.

Regulation of cytokine production by PPAR γ:

Cytokines produced by activated macrophages/foam cells, including the macrophage colony stimulating factor, IL-1, and TNF α, form the basis of the inflammatory component of the atherosclerotic lesions and promote proliferation of smooth muscle cells [7]. Necrosis of macrophages and lipid-loaded foam cells releases their intracellular contents, resulting in an accumulation of extracellular components that form the fibrous cap of the atheromatous lesion [16]. Eventually, the rupture of this plaque leads to the acute arterial obstruction [7]. Many aspects of these pathological processes might be modulated by PPAR γ which is upregulated by oxidized LDL [10, 20]. Furthermore, expression of PPAR γ has indeed been demonstrated in mouse and human atherosclerotic lesions [10, 11]. In contrast to this apparently proatherosclerotic action of PPAR γ ligands, these agonists have been reported to prevent atherosclerotic plaque progression [11]. Further studies are needed to determine the exact role of PPAR γ in the development of atherosclerosis.

Our studies revealed a positive relationship between anti-edema activity of PPAR γ agonists in vivo [19] and their ability to activate PPAR γ in vitro [8]. Thus, the high affinity PPAR γ agonist rosiglitazone, but not the low affinity agonist troglitazone, significantly inhibited paw edema [19]. This suggests that, like the PPAR α receptor, activation of the PPAR γ receptor leads to anti-inflammatory effects in vivo. Also, as with the PPAR α agonists, rosiglitazone was effective only when given prior to, but not after, carrageenan. Therefore, PPAR γ also appears to regulate the induction phase of inflammation. (Figures 2, 3)

Inhibition of Nitrotyrosine formation by PPAR γ:
Nitrotyrosine formed by a reaction of tyrosine residue with peroxynitrite, generated from superoxide and NO radicals. Suppression of iNO by PPARγ decreased the nitrotyrosine formation. Because iNO is an enzyme to produce NO during inflammatory conditions, PPARγ inhibits the nitrotyrosine formation in joint tissue.

**Role of PPARγ in neurodegenerative and autoimmune diseases:**

Release of inflammatory mediators has been postulated to play a major role in the etiology of a variety of aging-related neuronal degenerative diseases, such as Alzheimer’s disease (AD). The role of microglial-mediated inflammatory mechanisms in the etiology of AD has achieved prominence owing to recent compelling epidemiological and investigative findings. Epidemiological studies have shown a reduced risk of AD among long-term users of nonsteroidal anti-inflammatory drugs (NSAIDs). The formation of amyloid plaques in AD is accompanied by the recruitment of microglia to these deposits. The interaction of these cells with amyloid fibrils leads to their phenotypic conversion into a reactive phenotype. The activation of microglia results in the elaboration of a diverse array of pro-inflammatory secretory products including cytokines, chemokines, reactive oxygen species, and nitrogen species, as well as other acute phase proteins [32].

Previously, the anti-inflammatory actions of NSAIDs and their therapeutic benefit in treating AD were attributed to the ability of these drugs to inhibit the cyclooxygenases and PGE2 production [33]. Based on the findings showing that PPARγ ligands prevented the increase in Aβ-stimulated COX-2 expression in microglia and monocytes, it was determined that the neuroprotective effect of NSAIDs and PPARγ ligands was not attributable to a reduction in cyclooxygenase activity. This conclusion is supported by the fact that the COX-2-specific inhibitor NS-398 failed to promote neuron survival [32]. Furthermore, extended use of aspirin, a potent COX inhibitor, is not associated with a reduction in the risk of AD [33]. Consequently, it was concluded that microglial COX-2 activity and prostaglandin production are not necessary components in the neuronal death process, and that the beneficial effects of NSAIDs in AD are attributable principally to the actions of these drugs as PPAR agonists, rather than via their ability to inhibit cyclooxygenase activity [32].

Several studies have also investigated the role of PPARγ ligands in modifying animal models of autoimmune diseases. In a mouse model of inflammatory bowel disease, thiazolidinediones markedly reduced colonic inflammation. It was consequently proposed that this effect might be a result of a direct effect on colonic epithelial cells, which express high levels of PPARγ and can
produce inflammatory cytokines [34]. PPARγ ligands, 15d-PGJ2 and troglitazone, ameliorated adjuvant-induced arthritis with suppression of pannus formation and mononuclear cell infiltration in rats [35]. Niino et al [36] examined the effect of a thiazolidinedione on experimental allergic encephalomyelitis and found that these PPARγ agonists attenuated inflammation and decreased clinical symptoms in this mouse model of multiple sclerosis.

**CONCLUSION**

Demonstrating that PPAR agonists diminished inflammatory responses in several experimental models of inflammation has led to a surge in interest in these agonists as potential therapeutic agents to treat inflammatory diseases. Although various postulates have been advanced in an attempt to explain the mode of action of these compounds as anti-inflammatory agents, the exact mechanism by which they act remains elusive. Our recent results strongly suggest that agonists of PPAR α and PPAR γ interfere with the early phase of inflammation, without influencing its late phase. An unpublished finding from our laboratories shows that activation of PPAR δ also elicits an anti-inflammatory response. It is not yet clear, however, whether these three PPAR receptor subtypes share a common mechanism or act independently to control inflammatory processes.

PPARα and PPARγ decreased arthritis severity in adjuvant-induced arthritis. Both agonists partially protected animals from inflammatory induced-bone loss. PPARγ decreased the level of inflammatory bone destruction and protected the bone micro architecture in rats with AIA by controlling the circulating and local expression of IL-17.

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