New Development for Study of Oxidized Lipids
Guest Editor: Koji Uchida

Seeking for the Endogenous Ligands for PPARγ

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Summary Peroxisome proliferators-activated receptors gamma (PPARγ) is an isoform of PPARs which are members of the nuclear receptor superfamily and are considered as key sensors of lipid and glucose homeostasis. This ligand-activated transcription factor has been intensively studied for more than a decade and the bona fide endogenous ligand remains unknown. 15-Deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) is of great interest because it affects gene transcription by binding and activating PPARγ, and by covalent addition to transcription factors and signaling molecules. However, the actual concentration of 15d-PGJ2 is several orders of magnitude below the levels required to induce many of the biological reaction attributed to this molecule. This short review will focus on 15d-PGJ2 as a ligand of PPARγ, and on other candidates of the endogenous ligand for the receptor.

Key Words: 15d-PGJ2, PPARγ, ligand, transcription

The Roles of PPARs

Peroxisome proliferators-activated receptor (PPAR) is a nuclear hormone receptor that forms heterodimers with retinoid X receptor (RXR) and binds to direct repeat 1-type motifs found in the promoter sites of target genes [1]. The PPAR superfamily is composed of three isotypes, α, δ, and γ. These subtypes exhibit similar structure and sequence homology [2]. Most tissues have all three receptor subtypes, however, the relative expression is quite variable [3]. PPARγ is mainly expressed in the liver and regulates the expression of genes involved in the β oxidation of fatty acids [4] PPARγ is ubiquitously expressed in most mammalian tissues, and its function has yet to be precisely defined yet [5]. PPARγ is preferentially expressed in adipose tissue, colon and macrophages, then performs an important regulatory role in adipocyte differentiation and metabolism [6]. Like other nuclear receptors, the ligand-binding domains (LBD) of PPARs undergo encounters conformational changes when binding to specific ligands [7]. The DNA binding domain (DBD) is responsible for target gene recognition and DNA binding specificity is determined by its C-terminal extension as well as by receptor heterodimerization with the retinoid X receptor (RXR).

PPARγ was discovered in Xenopus [8] and mammals [9], and PPARγ exists in two protein isoforms (PPARγ1 and PPARγ2) that are created by alternative promoter usage and alternative splicing at the 5′ end of the genes. PPARγ2 contains 30 additional amino acids at the N terminus compared with PPARγ1 [1]. The major role of PPARγ is adipogenesis which is observed that ectopic expression of PPARγ in fibroblasts, preadipocyte, results in adipo-
The finding of a novel class of insulin-sensitizing drugs, the thiazolidinediones (TZDs), represents a new pharmacological class of oral antidiabetic agent. Before they were known to be ligands for PPARγ, TZDs were shown to be capable of initiating adipogenesis by Kletzien [11]. There is strong evidence that TZDs such as rosiglitazone function PPARγ to enhance insulin action by binding LBD with affinities in the nanomolar range. And the search for new activators of PPARγ represents a target of antidiabetic drug discovery.

15-Deoxy-Δ12,14-Prostaglandin J2 (15d-PGJ2), as a Ligand of PPARγ, and an Anti-Inflammatory PG In Vitro

PPARγ is required for adipogenesis [12] [10], and its agonists increase adipose mass in vivo as well as in vitro [13, 14]. Adipogenesis is a complex process associated with coordinated changes in gene expression, cell morphology, and hormone sensitivity [15], and several other transcription factors, such as CCAAT enhancer binding proteins (C/EBPs), are included.

Researchers’ interest grows in the 15d-PGJ2, a prostaglandin D2 derivative [16] independently, which was reported that it is able to bind to and activate PPARγ and induce adipogenesis. This was the first molecule to be identified as a possible endogenous PPARγ ligand and has been widely studied since then. Like other prostaglandins (PGs), 15d-PGJ2 can be actively transported into cells [17]. PGJ2 and its derivatives possess a highly reactive cyclopentenone (CP) ring [18] (Fig. 1). Independent of ligating to PPARγ, 15d-PGJ2 also covalently binds to certain proteins, such as glutathione, thioredoxin, and IκB kinase which inhibit the transcription factor nuclear factor-κB (NF-κB) [19, 20], which are the explanation for its pro- and anti-inflammatory action. All of which have directed towards the research assuming that 15d-PGJ2 exists in sufficient concentration for the biological efficacy.

Assay of 15d-PGJ2 by LC/MS and GC/MS

Like other prostaglandins, 15d-PGJ2 is measurable in biological samples, such as urine, synovial fluid as well as cultured medium by means of mass spectrometry [21, 22]. (Fig. 2) Urinary 15d-PGJ2 in healthy volunteers was 6.3 pg/mg creatinine, whereas another COX product, such as prostacycline's...
metabolite, 2,3-dinor-6-keto-PGF$_{10}$, was over 20 times more exist. Although there are many investigations assuming that 15d-PGJ$_2$ plays a major role in many pathological conditions, such as obesity, type 2 diabetes, inflammation, and atherosclerosis, there was no significant alteration in biosynthesis in those conditions [27]. From the experiments of in vivo COX inhibitors, COX-2 is the dominant source of 15d-PGJ$_2$ biosynthesis under physiologic conditions in human.

**Seeking for the Endogenous Ligand of PPAR$_{\gamma}$**

The role of PPAR$_{\gamma}$ in the aforementioned cellular and metabolic processes has been well established in this decade. Many investigators have approached to the provocative issue that what is the true ligands of PPAR$_{\gamma}$, (Fig. 3). The reason for that is because finding the *bona fide* endogenous ligand will advance our understanding of PPAR$_{\gamma}$ modulation and reveal the mechanism of diverse metabolic disorders. However, Genetic variation, structural features and studies from knockouts of PPAR$_{\gamma}$ have complicated the issue.

According to the analysis of crystal structure of PPAR$_{\gamma}$, LBD, the LBD of PPAR$_{\gamma}$ has 13 α helix and small four-stranded β sheet. The PPAR$_{\gamma}$ ligand-binding site is a large T shaped cavity and also interactions of PPAR$_{\gamma}$, with coactivators is ligand type-specific. For example, direct interactions of PPAR$_{\gamma}$, with TRAP220 were observed when 15d-PGJ$_2$ but not when troglitazone was bound [23]. Those structural analyses gave us the possibility that the endogenous PPAR$_{\gamma}$ ligand may be more than one and differs in target tissue. Or it may consist of two or more different molecules and the combination interact with its LBD and regulate the recruitment of coactivators and the consequent response. For example, some isoprostanes shows low affinity to activate the receptor each and combination of two different isoprostanes produce more than each of the maximum response [24]. Another structural analysis revealed that the α, β-unsaturated ketone enables the ligands to bind to PPAR$_{\gamma}$ covalently and to exhibit their activity. This also suggests that PPAR$_{\gamma}$ may function under oxidative conditions and/or inflammation [25].

Some fatty acids including arachidonate and linoleate and their oxygenated metabolites including 9-HODE, 13-HODE bind and activate PPAR$_{\gamma}$, [26]. But because all of their $K_D$ values are in the same low micro molar range [27] they are not likely to function as endogenous ligands in physiological condition. Although some prostaglandins and its deriva-
Ligands of PPARγ

<table>
<thead>
<tr>
<th>Synthetic ligands</th>
<th>Natural ligands</th>
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<tr>
<td>Thiazolidinedione (TZD); rosiglitazone</td>
<td>Polynsaturated fatty acids (PUFAs)</td>
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<tr>
<td></td>
<td>linoleic acid</td>
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<td></td>
<td>arachidonic acid</td>
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<td>2-100 μM</td>
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<tr>
<td>ciglitazone</td>
<td>Eicosapentanoic acid</td>
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<td>9-HODE</td>
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<td>13-HODE</td>
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<td>15-HETE</td>
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<td>10-20 μM</td>
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<tr>
<td>troglitazone</td>
<td>15-deoxy-Δ12,14-PGJ2</td>
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<tr>
<td>WY14643</td>
<td>Hexadecyl azelaoyl</td>
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<tr>
<td>ETYA</td>
<td>phosphatidylycholine (azPC)</td>
</tr>
<tr>
<td>indomethacin</td>
<td>~40 nM</td>
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<tr>
<td>FMOC-L-leucine</td>
<td>Lyosphosphatic acid (LPA)</td>
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<tr>
<td>(FLL)</td>
<td>Nitrolinoleic acid (LNO2)</td>
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Fig. 3. Synthetic and natural ligands of PPARγ.

tives are proposed as PPARγ ligands, their intrinsically low binding affinities and/or insufficient *in vivo* concentration do not support the capability to serve as physiologically relevant signaling mediators.

McIntyre *et al.* [28] has reported that free radical oxidation of low-density lipoprotein (LDL) fragments its phospholipids, and one of these phospholipids oxidation products, hexadecyl azelaic phosphatidylycholine (azPC), binds and activates PPARγ, as potently as rosiglitazone. But to generate this putative endogenous PPARγ agonist, aggressive oxidizing conditions *in vitro* are necessary.

Lysosphosphatic acid (LPA) is another PPARγ ligand which stimulates PPARγ-responsive element and endogenous PPARγ-controlled gene CD36 [29]. But the binding affinity has not been determined yet.

Recently, Schopfer *et al.* has reported that nitrolinoleic acid (LNO2) binds and activates PPARγ [30]. This most abundant bioactive oxide of nitrogen which exists as 80 nM free and 550 nM esterified LNO2 form and the fatty acid nitration products may transduce NO-mediated cell signaling via PPARγ activation. It will be of great interest if the concentration of LNO2 alters in many clinical settings which are suggested that PPARγ is relevant, such as diabetes, obesity, and inflammation.

The mechanism of adipocyte differentiation and metabolism, inflammation, and cancer regulated by PPARγ is of great interest. Still the overall effect of PPARγ remains unknown in some disease, so that identifying endogenous ligands may contribute to better understanding of the biological role of regulation of PPARγ, activity.

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References


