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Review

The role of nitrated fatty acids and peroxisome proliferator-activated receptor gamma in modulating inflammation

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ABSTRACT

Nitrated fatty acids (NFAs), thought to be produced by nonenzymatic reactions of endogenous nitric oxide (NO) with naturally present unsaturated fatty acids, have recently been identified as one of the largest single pools of biologically active NO derivatives in human plasma. As the biological role of NFAs is unknown, initial *in vitro* studies have shown them to be potent suppressors of inflammatory responses. The aim of the study was to collect all the literature on NFAs and its interactions with peroxisome proliferator-activated receptor gamma (PPAR-γ) and review in detail the anti-inflammatory properties of PPAR-γ interceded by NFAs. A literature survey was performed using PubMed and ScienceDirect to gather complete information on NFAs and their interactions with PPAR-γ. An exhaustive literature survey revealed that NFAs found in human plasma and urine comprises a class of cell signaling mediators that can activate PPAR-γ within its physiological concentration. NFAs exhibit anti-inflammatory and anti-fibrotic effects through PPAR-γ activation in various *in vitro* models tested. Besides its role in inflammation other properties of NFAs such as inhibition of enzymes, inducer of gene expression, *etc.*, were discussed. NFAs are good electrophiles with pleiotropic biological activities. Hence NFAs can be treated as potent drug candidates.

Contents

1. Introduction

Fatty acids are monocarboxylic, long chain aliphatic compounds which are usually obtained *via* diet or derived from endogenous triacylglycerol and phospholipids. Most naturally occurring fatty acids contain an even number of aliphatic carbons (4–28 carbons) and may be either saturated or unsaturated [1]. In their usual form fatty acids are an important source of energy. Upon modification, for instance nitration, fatty acids become potent bio-signals [2]. Nitrated fatty acids (NFAs) are a subset of fatty acids that are produced in myriad pathways. An oxygen rich, hydrophobic environment provides a favorable condition for

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the formation of NFAs. It is understood that fatty acids are nitrated through a process similar to that of proteins and DNA bases [3]. Fatty acid nitration occurs by any one of the following reactions: [4,5]

1. Peroxynitrite ($ONOO^-$) mediated addition which is predominant in inflammatory cells.
2. Peroxidase catalyzed additions.
3. Nitrous acid (HNO_2) mediated addition which is common in acidic conditions prevailing in gastric cells. A sizable amount of fatty acids taken through diet is nitrated by this reaction. This reaction is also important in phagocytic cells that are known to be acidic by possessing a large number of acidic compartments like lysosomes and endosomes.
4. Direct nitration of fatty acids in which a high amount of reactive nitrogen species (RNS) concentrates in membranes due to the prevailing hydrophobic conditions termed as "lens effect".

Practically, among fatty acids, polyunsaturated fatty acids (PUFA) were mostly nitrated due to its abundance [5,6]. Detailed chemical mechanisms of nitration of specific fatty acids can be found elsewhere [7,8]. Nitrated macromolecules lose their NO readily in polar solvents and the released NO may still transduce signals *via* cGMP [9]. This is true for fatty acids also [10] but ironically most of the NFAs by their prolonged half-life [2] were destined to become chemical messengers by regulating proteins, particularly transcription factors.

NFAs are excellent electrophiles that can act either independently or in combination with other macromolecules, namely proteins to bring about their consequences. Irreversible inhibition of xanthine oxidase (XO) exemplifies the independent effect [11]. On the other hand, NFAs are endogenous peroxisome proliferator-activated receptor gamma (PPAR-γ) ligands [12].

PPARs are nuclear receptors that require ligands for activation. PPARs are principal lipid sensing agents that play a vital role in metabolic homeostasis. PPARs comprise three isotypes, namely α, β/δ, and γ [13]. Activation of PPAR-γ by certain ligands leads to differentiation of preadipocytes into adipocytes [14] and inactivation of macrophages [15]. To a small extent other leukocytes like neutrophils, dendritic cells, T-cells, and B-cells are affected [16]. Thiazolidinediones (TZD), a class of synthetic anti-type 2 diabetic drugs, were the first compounds found to activate PPAR-γ [17]. Most putative endogenous PPAR-γ ligands to date were not found in sufficient quantity to elicit PPAR-γ with some prostaglandins as exceptions [18]. A complete list of putative endogenous PPAR-γ ligands can be found elsewhere [19]. NFAs were found to bind PPAR-γ with affinities rivaling that of synthetic high affinity TZD. Under certain circumstances, NFA concentrations may reach >1 μM, much higher than its effective concentrations of PPAR-γ activation (100 nM). The binding is also unique such that the other PPAR isotypes seldom bind NFAs (~300 nM) [20,21]. Hence, it is clear that NFAs may interact with PPAR-γ *in vivo*. We must understand the molecular mechanisms of NFA formation and the paradigms involved in PPAR-γ activation in order to appreciate consequences brought about by them.

All PPAR isoforms bind to and are activated by fatty acids containing >12 carbon atoms [22]. While PPAR-α and to a lesser extent PPAR-β/δ bind saturated, hydrophobic fatty acids, PPAR-γ prefers more hydrophilic ligands. Apart from NFAs, PPAR-γ binds polyunsaturated, oxidized, and charged fatty acids [23]. A closer look into the Y-shaped, about 1300 Å big ligand binding pocket of PPAR-γ justifies this phenomenon. Structures of PPAR-γ co-crystallized with NFAs reveal that the position of NO_2 and charged amino acids, Arg288 and Glu343 in PPAR-γ, are critical for the binding of these two molecules. Arg288 and Glu343 are unique to PPAR-γ among the highly conserved ligand binding domain of PPARs. This is the reason for NFAs to selectively recognize PPAR-γ. Mutating Arg288 and/or Glu343 to a hydrophobic amino acid resulted in non-recognition of the receptor by NFAs, while conversion of hydrophobic amino acids in a ligand binding pocket to hydrophilic amino acids enhanced PPAR-γ activation. These mutations did not affect the binding or receptor activation of TZD [24]. Thus, understanding PPAR-γ–NFA interactions may open new vistas in PPAR-γ biology. The following text of the review is intended to give more information on PPAR-γ–NFA mediated effects in inflammation, recent advances in this field, and future directions for understanding endogenous activation of PPAR-γ. Other properties of NFAs such as inhibition of enzymes, and inducer of gene expression were also discussed.

2. PPAR-γ mediated anti-inflammatory properties of NFAs

PPAR-γ is the principal regulator of insulin signaling, adipocyte differentiation, and inactivation of macrophages. Upon activation by ligands, PPAR-γ heterodimerizes with retinoid-X-receptor and translocates into the nucleus to bind a portion of DNA called PPAR response elements (PPRE) that bring about transcription of downstream genes. Lipoprotein lipase, CD36, phosphoenolpyruvate carboxykinase, aquaporin-7, and adiponectin are the adipogenic genes under the control of PPAR-γ. Simultaneously, expression of certain proteins was blocked by PPAR-γ due to its trans-repressor property. Some of the downregulated genes include a-disintegrin and metallopeptidase domain-8 (ADAM8), macrophage inflammatory protein-1α (MIP-1α), macrophage antigen-1 (MAC-1), F4/80+, and CD68 [25,26]. PPAR-γ was shown to downregulate the expression of STAT, NF-κB, and AP-1 [27]. Activated PPAR-γ will directly bind corepressors of these proteins, making them unfit for nuclear translocation. This process shuts down the transcriptional activity of the aforementioned transcription factors and may nullify symptoms of inflammation [28]. Anti-inflammatory properties of PPAR-γ are extensively reviewed elsewhere [16,29,30].

NFAs were found to be excellent high affinity ligands ($K_d \leq 200$ nM) of PPAR-γ second only to synthetic TZD ($K_d \leq 100$ nM) [20]. For instance, nitrolinoleate strongly activated PPAR-γ dependent transcription in a study using MCF-7 cells transiently transfected with PPRE reporter gene construct [31]. *In vivo* administration of an NFA, nitrooleic acid, which is a PPAR-γ agonist to diabetic Zucker rats showed that it ameliorated all diabetic symptoms without much side effects [32]. There are evidences of PPAR-γ binding its ligands covalently by Michael's addition [33]. Covalent attachment of NFA activated PPAR-γ only partially. Hence, NFAs may be used which are better than TZD in treating diabetes in terms of side effects [34]. Most of the putative endogenous PPAR-γ ligands were those found in an oxidizing and inflammatory milieu. NFAs, which are also formed under inflammatory conditions, activate PPAR-γ in different ways.

An NFA, 10-nitro-oleate was reported to activate PPAR-γ protected LPS-induced endotoxemia and acute lung injury [35,36]. NFA bound PPAR-γ ameliorated dextran sulfate sodium-induced inflammatory bowel disease in mice by abolishing colon shortening [37]. In a recent study, Lakshmi and coworkers [38] demonstrated that activation of PPAR-γ by nitro-oleic acid in human lung epithelial cells (treated with cigarette smoke extracts (CSE)), upregulated PPAR-γ expression which in turn completely reversed the CSE-mediated effects on anti-inflammatory proteins and proinflammatory transcription factor NF-κB, cytokine, chemokine, and ROS production. These results suggest that activation of PPAR-γ by its ligands, particularly by endogenous NFAs, plays a pivotal role in amelioration of chronic obstructive pulmonary disease (COPD) and further suggests that PPAR-γ agonists may be useful for the treatment of COPD.

10-Nitrooleate supposedly enhanced the inhibitory activity of PPAR-γ, and improved apoptosis of neutrophils and their phagocytosis by alveolar macrophages at a level significantly higher than the steroid drug fluticasone. Hence, 10-nitrooleate may be used to treat advanced, steroid resistant asthma [39]. Suppression of TNF-α by NFAs followed by subsequent activation of PPAR-γ resulted in the inhibition of monocyte–endothelial cell adhesion function. Many of the proinflammatory cytokines like interleukins (IL-6, IL-8, IL-12/p40), IFNc, MCP-1, and IP-10 and cell-adhesion molecules including ICAM and VCAM secreted in response to TNF-α were concomitantly downregulated [40].