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Review

Inflammatory signaling and metabolic regulation by nitro-fatty acids

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ABSTRACT

The addition of nitrogen dioxide (·NO_2) to the double bond of unsaturated fatty acids yields an array of electrophilic nitro-fatty acids (NO_2-FA) with unique biochemical and signaling properties. During the last decade, NO_2-FA have been shown to exert a protective role in various inflammatory and metabolic disorders. NO_2-FA exert their biological effects primarily by regulating two central physiological adaptive responses: the canonical inflammatory signaling and metabolic pathways. In this mini-review, we summarize current knowledge on the regulatory role of NO_2-FA in the inflammatory and metabolic response *via* regulation of nuclear factor kappa B (NF-κB) and peroxisome proliferator-activated receptor γ (PPARγ), master regulators of inflammation and metabolism. Moreover, the engagement of novel signaling and metabolic pathways influenced by NO_2-FA, beyond NF-κB and PPAR signaling, is discussed herein.

1. Introduction

Endogenous nitro-fatty acids (NO_2-FA) are generated during inflammation and digestion through non-enzymatic reactions of unsaturated fatty acids with nitrogen dioxide (·NO_2) [1]. Conjugated linoleic acid (CLA) is the preferred fatty acid target of nitration reactions due to the highly reactive external flanking carbons in the conjugated diene, yielding electrophilic nitro-conjugated linoleic acid (NO_2-CLA), the predominant endogenous isomer detected *in vivo* [2].

A large body of evidence has accumulated during the last decade supporting a protective role for NO_2-FA in numerous experimental settings [3]. These include endotoxin-induced vascular inflammation, endotoxemia and multi-organ injury [4,5], inflammatory bowel disease (IBD) [6], allergic airway disease [7], renal ischaemia and reperfusion (I/R) injury and diabetic kidney disease [8,9], pulmonary arterial hypertension (PAH) [10,11], myocardial I/R injury [12], hypertension [13,14], and atherosclerosis [15]. Beyond the above experimental models, the safety and pharmacokinetics of NO_2-FA have been clinically examined in four successfully completed phase I trials (NCT02127190, NCT02248051, NCT02460146, NCT02313064) [16–19]. Currently, NO_2-FA administration is entering phase II clinical trials for the treatment of focal segmental glomerulosclerosis (FSGS), PAH and obese asthmatics.

The above protective effects on NO_2-FA are meditated by their pluripotent cell signaling capabilities, affecting various intracellular pathways. First, NO_2-FA modulate nitric oxide (·NO) signaling by yielding low concentrations of ·NO (*via* Nef reaction) that mediate cGMP-dependent cell signaling activities and *via* a non cGMP-dependent manner in which NO_2-FA regulate endothelial and inducible nitric oxide synthase (eNOS and iNOS)-mediated ·NO generation and reactions [20–22]. Second, NO_2-FA can potently regulate the expression of key inflammatory, cell proliferation and differentiation-related genes [4,23–27]. Third, NO_2-FA are endogenous ligands for peroxisome proliferator-activated receptors (PPARs), mainly PPARγ, centrally involved in lipid and glucose homeostasis as well as inflammation [28–30]. Finally, NO_2-FA facilitate reversible adduction by nucleophilic targets (e.g. Cys and His protein residues), leading to the post-translational modifications (PTM) of proteins [31,32]. Particularly, the electrophilic nature of NO_2-FA results in nitroalkylation of nuclear factor kappa B (NF-κB), the master regulator of the immune and inflammatory response, inhibiting its DNA binding activity and repressing inflammatory gene expression, underlying a key role for NO_2-FA as endogenous anti-inflammatory signaling mediators [24]. This mini-review focuses on NO_2-FA regulation of NF-κB and PPARγ, key regulators of inflammation and metabolism, and the implication for inflammatory and metabolic disorders.

2. NO_2-FA promote cellular anti-inflammatory responses *via* NF-κB inhibition

NO_2-FA exerts potent anti-inflammatory actions, primarily by antagonizing the activities of NF-κB, and signal transducers and activators

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Abbreviations

CLA	conjugated linoleic acid
eNOS	endothelial nitric oxide synthase
IBD	inflammatory bowel disease
iNOS	inducible nitric oxide synthase
NCoR	Nuclear receptor co-repressor
NF-κB	nuclear factor kappa B
·NO	nitric oxide;
NO_2^-	nitrite;
·NO_2	nitrogen dioxide;
NO_2-FA	nitro-fatty acids
NO_2-OA	nitro-oleic acid
NO_2-LA	nitro-linoleic acid
NO_2-CLA	nitro-conjugated linoleic acid
PAH	pulmonary arterial hypertension
PPARγ	peroxisome proliferator activating receptor γ
PTM	posttranslational modification
TZDs	thiazolidinediones

of transcription (STATs) [25,33], while activating PPARγ and the anti-inflammatory nuclear factor E2-related factor 2 (Nrf2) [23,28,34]. Yet, a key mechanism behind the role of NO_2-FA is their ability to inhibit NF-κB activity by preventing the phosphorylation of inhibitor of kappa B (IκB) and its subsequent degradation as well as by activating PPARγ (Fig. 1). Studies using affinity labeling and mass spectrometry strategies reveal that NO_2-FA derivatives inhibit NF-κB *via* nitroalkylation of Cys residues in the NF-kB p65 subunit *in vitro* and a similar reaction with p65 in macrophages treated with NO_2-FA [24]. Both nitro-oleic acid (NO_2-OA) and nitro-linoleic acid (NO_2-LA) or novel NO_2-FA derivatives inhibit lipopolysaccharide (LPS)-induced macrophage secretion of pro-inflammatory cytokines including interleukin 6 (IL-6), tumor necrosis factor α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) as well as iNOS expression [24,35,36]. NO_2-FA down-regulate expression or activity of pro-inflammatory signaling molecules induced by various stimuli (e.g., TNF-α, LPS, IL6, TGF-β) [24,26].

Further experimental evidence reveals that NO_2-FA regulate the NF-κB signaling pathway at multiple levels (Fig. 1). These include reduced membrane expression of Toll-like receptor 4 (TLR4) and impaired recruitment of TLR4 and TNF receptor associated factor 6 (TRAF6) to the lipid rafts compartment with subsequent inhibition of IκB kinase β (IKKβ) phosphorylation as well as phosphorylation and ubiquitination of IκB-α, [4]. In addition, alkylation the NF-κB p65/RelA by NO_2-FA, targeting RelA for proteasomal degradation has been demonstrated in triple negative breast cancer (TNBC) cells [37].

The anti-inflammatory properties of NO_2-FA *via* NF-κB suppression resemble that of known NF-κB inhibitors. For instance, TNBC cells treated with NO_2-OA or the IKKβ inhibitor 3-[(4-methylphenyl)sulfonyl]-(2E)-propenenitrile (Bay 11-7082) show a similar inhibitory effect on TNFα-induced IKKβ phosphorylation and IκB degradation [37]. Also, the oleanane triterpenoid 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid methyl ester (CDDO-Me, Bardoxolone methyl) contains α,β-unsaturated carbonyl in the A-ring that forms reversible adducts with thiol nucleophiles. CDDO-Me inhibits the NF-κB pathway by interacting

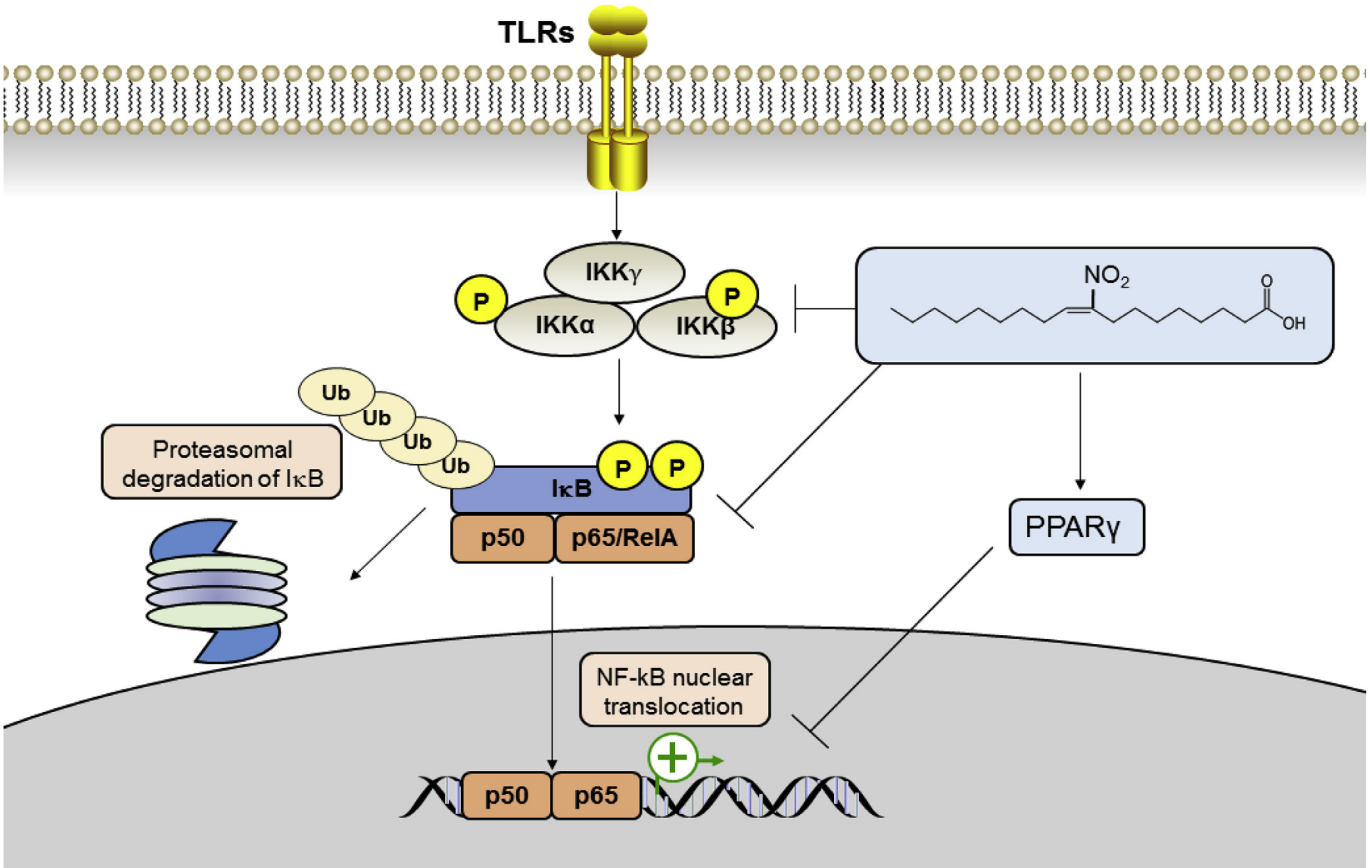

Fig. 1. NO_2-FA promote cellular anti-inflammatory responses through NF-κB suppression *via* inhibition of IκB phosphorylation and its subsequent degradation, PTM (e.g. p65 nitroalkylation) or PPARγ transrepression signaling. NO_2-FA-induced NF-κB inhibition contributes to prevention of vascular inflammation and endotoxemia, allergic airway disease, myocardial I/R injury and myocardial fibrosis [3].