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Anti-inflammatory signaling actions of electrophilic nitro-arachidonic acid in vascular cells and astrocytes☆

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ABSTRACT

Nitrated derivatives of unsaturated fatty acids (nitro-fatty acids) are being formed and detected in human plasma, cell membranes and tissue, triggering signaling cascades *via* covalent and reversible post-translational modifications of nucleophilic amino acids in transcriptional regulatory proteins. Arachidonic acid (AA) represents a precursor of potent signaling molecules, i.e., prostaglandins and thromboxanes through enzymatic and non-enzymatic oxidative pathways. Arachidonic acid can be nitrated by reactive nitrogen species leading to the formation of nitro-arachidonic acid (NO_2-AA). A critical issue is the influence of NO_2-AA on prostaglandin endoperoxide H synthases, modulating inflammatory processes through redirection of AA metabolism and signaling. In this prospective article, we describe the key chemical and biochemical actions of NO_2-AA in vascular and astrocytes. This includes the ability of NO_2-AA to mediate unique redox signaling anti-inflammatory actions along with its therapeutic potential.

1. Introduction

Free as well as esterified fatty acids are important components of biological membranes that can be modified by reactive oxygen (ROS) and nitrogen (RNS) species. Nitration of fatty acids is expected to occur in hydrophobic compartments such as the lipid bilayer of cellular membranes or the lipophilic core of lipoproteins. It is well reported that RNS both oxidize and nitrate unsaturated fatty acids yielding an array of hydroxyl, hydroperoxy, nitro and nitrohydroxy derivatives [1], with the *in vivo* mechanism for nitro-fatty acid (NO_2-FA) formation still remain unknown. Most of the available work suggests the presence of nitrogen dioxide ($^{\bullet}NO_2$) as the limiting step, being its most probable source nitric oxide ($^{\bullet}NO$) autooxidation [2]. Also, $^{\bullet}NO_2$ can be generated from the decomposition of peroxynitrite, peroxidase-catalyzed oxidation of nitrite (NO_2^-) to $^{\bullet}NO_2$ or reduction of NO_2^- in acidic tissue environments (e.g., gastric compartment, ref. [3]). In fact, $^{\bullet}NO$ and superoxide radical ($O_2^{\bullet -}$) are the precursors of peroxynitrite which under inflammatory conditions where it is observed an increase of either oxidized and nitrated proteins and lipids [4]. Since both peroxynitrite and $^{\bullet}NO_2$ readily diffuse through the membrane bilayers, reactions leading to $^{\bullet}NO_2$ generation may take place in the aqueous environment in proximity to the membrane or inside the lipid bilayer [5].

Inflammatory related diseases (e.g. atherosclerosis, diabetes) present an increase of both ROS and RNS formation as well as the participation of macrophages and platelets in many of the pathological states derived from the activation of inflammatory processes [6–9]. Both cell types have an active participation of arachidonic acid (AA) metabolizing pathway with the formation of pro- and anti-inflammatory eicosanoids as well as formation of ROS and RNS and changes in the cell redox status [10–13]. The aim of this review is to discuss the biological effects of the addition of a nitro group (-NO_2) to the carbon chain of AA in platelets, macrophages and astrocytes. Modulation of pro- and anti-inflammatory enzymes by nitroarachidonic acid (NO_2-AA) as well as key cellular signaling pathways are detailed.

1.1. Enzymatic oxidation of arachidonic acid

Arachidonic acid (20:4) is the precursor of many biologically- and physiologically-relevant bioactive lipids, being its cellular

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metabolism regulated from its synthesis through AA release and transport from cell membranes by phospholipase A_2 [14]. At the cytosol, AA can be oxidized enzymatically and non-enzymatically leading to the formation of pro- and anti-inflammatory derived prostanoids [14]. Formation of bioactive lipids derived from AA at inflammation sites are well regulated by basal metabolism as well as by cytokines [15]. Moreover, final destination of AA depends on the tissue content of the enzymes capable of oxidize AA and its derivatives. For example, the same precursor prostaglandin H_2 (PGH_2) is transformed into thromboxane A_2 (TxA_2) in platelets while is oxidized to prostacyclin (PGI_2) in endothelial cells, having TxA_2 and PGI_2 opposite effects on platelet aggregation [15,16].

Prostaglandin endoperoxide H synthase (PGHS) is the key enzyme of AA metabolism as it final oxidation product, PGH_2, is the precursor of various lipid mediators, e.g. prostaglandins, thromboxanes. The enzyme catalyses the two step transformation of AA into PGH_2: dioxygenation of AA to prostaglandin G_2 (PGG_2) performed by the cyclooxygenase (COX) reaction and the subsequent reduction of PGG_2 to PGH_2 by the peroxidase (POX) reaction [17]. Both activities require the non-covalent attached heme to the enzyme. Inflammatory processes and polyunsaturated fatty acids (PUFAs) are linked by eicosanoids, which represent mediators and regulators of inflammatory processes formed from 20 carbon length-PUFAs. PGHS enzymes are target for the inhibitory actions of non-steroidal anti-inflammatory drugs (NSAIDs) [15].

In contrast to PGHS, lipoxygenase (LOX) is a non-heme iron containing dioxygenase that catalyses the direct reaction of oxygen with AA [15,16]. Fatty acid oxidation by LOX starts by abstracting a hydrogen atom from the carbon chain in a stereospecific manner, followed by radical rearrangement and oxygen insertion leading to the formation of hydroperoxides, which in the case of AA corresponds to hydroperoxy eicosatetraenoic acid (HPETE) [15,16]. The HPETE products once formed are reduced by cellular peroxidases to the bioactive lipid, hydroxyeicosatetraenoic acid (HETE). Many mammal tissues present different LOX isoforms named in accordance to the carbon atom to whom the hydroperoxides group is attached, i.e. 5-LOX, 12-LOX, 15-LOX [18–21]. Importantly, while aspirin or other PGHS inhibitors inhibit PGHS, LOX remain active being able to oxidize AA to HPETEs [15,16]. The different isoforms are distributed in different tissues and cell locations. Reports show that LOXs are the first committed step in a cascade of metabolic pathways implicated in the onset of inflammatory diseases making the enzyme an ideal candidate for drug development [18,22,23].

The effects of their products on different tissues also evidence the importance of both PGHS and LOX in AA metabolism. In particular the formation of the PGHS-derived product TxA_2 in platelets led to platelet aggregation while the effects of 12-HETE due to 12-LOX still not clear. There are reports showing that metabolic products of 12-LOX attenuate AA-induced aggregation [24] as well as decrease the release of AA from membrane phospholipids [25], whereas others propose that 12-LOX activation is prothrombotic [26]. Taking into account the relevance of AA signaling pathway in vascular cells as platelets and macrophages, as well as the differential outcome of the bioactive lipids formed in different tissues the effects of the nitrated derivative of AA (NO_2-AA) will be discussed separately in platelet and macrophages.

1.2. Interaction of NO_2-AA with lipid metabolizing enzymes in platelets

Nitroarachidonic acid (NO_2-AA) can be formed in biological membranes from the AA present in the 2-carbon position of phospholipids. Phospholipase A_2 (PLA_2) hydrolyze fatty acids from the sn-2 position of phospholipids at the cell membranes releasing free fatty acids (e.g. AA), being the precursors for a wide group of signaling molecules. The released AA, following PLA_2 activity, is a substrate for nitration reactions leading to NO_2-AA formation.

The presence of nitrated derivatives of AA was first reported as nitrohydroxyarachidonic acid (NO_2-(OH)-AA) in bovine cardiac muscle [27]; years later both NO_2-AA and NO_2-(OH)-AA were detected in human plasma and urine [28]. However, none of this studies determined which were the main isomers formed in those experimental conditions. We performed chemical synthesis and preparation of NO_2-AA derivatives [29]. Firstly, a complex mixture of products has been identified after reaction of AA with peroxynitrite/$^{\bullet}NO_2$ including *cis-trans* isomerization and formation of nitro-hydroxy arachidonate (Fig. 1) [27]. Hydrophobic membranes may facilitate reactions where $^{\bullet}NO_2$, arachidonyl (AA$^{\bullet}$) and arachidonyl peroxyl (AAOO$^{\bullet}$) radicals are likely to be simultaneously present. Thus, biological effects of NO_2-AA have been performed using a mixture of four mononitrated nitroalkenes: 9-nitroicosa-5,8,11,14-tetraenoic acid (9-NO_2-AA), 12-nitroicosa-5,8,11,14-tetraenoic acid (12-NO_2-AA), 14-nitroicosa-5,8,11,14-tetraenoic acid (14-NO_2-AA) and 15-nitroicosa-5,8,11,14-tetraenoic acid (15-NO_2-AA) [29]. These isomers were obtained from chemical synthesis and reaction batches contained in all cases a mixture of 23% of 12- and 15-NO_2-AA, 55% of 9-NO_2-AA and 22% of 14-NO_2-AA [30].

As explained before, AA is the substrate for PGHS activity thus its nitration may divert the fatty acid from its normal metabolizing

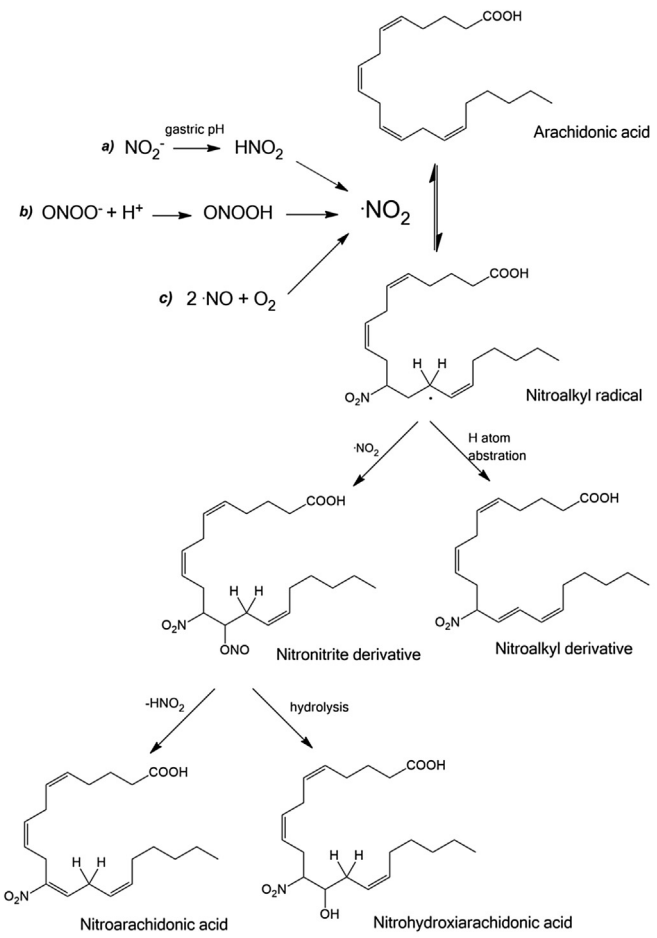

Fig. 1. Arachidonic acid nitration. Arachidonic acid can be nitrated by nitrogen dioxide ($^{\bullet}NO_2$) coming from a) nitrite under acidic conditions; b) $^{\bullet}NO$ autooxidation or c) peroxynitrite homolysis. Reactivity of $^{\bullet}NO_2$ led to nitroalkyl- (nitroalkanes), nitro-hydroxy- (NO_2(OH)-) or nitroalkene- (NO_2-AA) derivatives.

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