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Antagonism of human formyl peptide receptor 1 with natural compounds and their synthetic derivatives

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ABSTRACT

Formyl peptide receptor 1 (FPR1) regulates a wide variety of neutrophil functional responses and plays an important role in inflammation and the pathogenesis of various diseases. To date, a variety of natural and synthetic molecules have been identified as FPR1 ligands. Here, we review current knowledge on natural products and natural product-inspired small molecules reported to antagonize and/or inhibit the FPR1-mediated responses. Based on this literature, additional screening of selected commercially available natural compounds for their ability to inhibit *f*MLF-induced Ca^{2+} mobilization in human neutrophils and FPR1 transfected HL-60 cells, and pharmacophore modeling, natural products with potential as FPR1 antagonists are considered and discussed in this review. The identification and characterization of natural products that antagonize FPR1 activity may have potential for the development of novel therapeutics to limit or alter the outcome of inflammatory processes.

1. Introduction

Forty years ago, Schiffmann et al. [1] reported that *N*-formylated peptides are potent chemotactic agents for human neutrophils. Further study of the biological targets of formylated peptides led to the identification and subsequent cloning of human formyl peptide receptor 1 (FPR1) [2,3]. Two other relatively conserved low-affinity *f*MLF receptors, now termed as FPR2 and FPR3, were subsequently cloned (reviewed in [4]). FPR1 is a key regulator of the inflammatory environment. However, the expression of FPRs in various nonphagocytic cells suggests that these receptors also participate in functions other than innate immunity and may represent unique targets for therapeutic drug design [5–7]. Such drugs may have the potential to treat many inflammatory diseases, including rheumatoid arthritis, asthma, other autoimmune diseases, and stimulate wound healing [6,8–10].

Since FPRs represent potentially important therapeutic targets, much attention has been focused over the last two decades on the identification of natural and synthetic compounds that interact with these receptors and/or interfere with FPR-dependent pathways. To date, several reviews summarizing the research efforts on FPR1 and FPR2 agonists, including natural ligands, have been published [4,11–16]. However, less attention has focused on natural FPR1 antagonists.

Natural products traditionally have played an important role in drug discovery and were the basis of most early medicines [17]. The potential of natural products as sources for new drugs is still largely unexplored, and only a small fraction of the products present in existing plants, fungi, microorganisms, and animals have been investigated so far. Previously, we reported that many FPR1 antagonists contain OH groups, which can serve as H-bond donors and/or acceptors upon binding to the receptor, and that this feature is much more characteristic of FPR1 antagonists than agonists [16]. Because natural compounds in general incorporate more oxygen atoms than synthetic compounds and drugs [18], this feature makes them attractive for screening as FPR1 antagonists. Furthermore, natural products contain more fused rings, but fewer rotatable bonds than synthetic medicinal compounds [18], and this particular feature is also characteristic of FPR1 antagonists [16,19].

FPR1 activation stimulates multiple signal transduction pathways responsible for various neutrophil functions, such as adhesion, chemotaxis, phagocytosis, exocytosis of secretory granules, and superoxide anion radical ($O_2^{-\bullet}$) production, which contribute to the physiological

Abbreviations: Cs, Cyclosporine; Cs, Cyclosporine; CHIPS, chemotaxis inhibitory protein of *Staphylococcus aureus*; FPR, formyl peptide receptor; GPCR, G-protein coupled receptor; HNE, human neutrophil elastase; PAF, platelet activating factor; PMA, phorbol-12-myristate-13-acetate; ROS, reactive oxygen species; SAR, structure–activity relationship; FITC, fluorescein isothiocyanate; NDGA, nordihydroguaiaretic acid.

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inflammatory response associated with bacterial infection and tissue damage (reviewed in [4,20,21]). The main molecular events and mediators involved in FPR1 activation are summarized in Fig. 1. The FPR1 ligand *f*MLF is known to activate phospholipases C (PLC) and A_2 (PLA_2) and release of intracellular Ca^{2+} stores. The second messengers resulting from FPR1 activation regulate various intracellular kinases, including phosphatidylinositol-3-kinase (PI3K), protein kinase C (PKC), and mitogen-activated protein (MAP) kinases p38 and extracellular signal related kinase 1 and 2 (ERK 1/2). Activation of FPR1 stimulates an additional protein kinase C-independent pathway through the Src-related tyrosine kinase, Lyn, in human neutrophils [22]. Likewise, downstream activation of Rho GTPases, particularly Rac1 and Rac2, plays a key role in neutrophil NADPH oxidase assembly, chemotaxis, and degranulation [23]. Activation of these signal-transduction pathways is known to be responsible for the various biochemical responses that contribute to physiological defense against pathogens and the inflammatory response to cellular damage. Although the intrinsic functional redundancy in the chemoattractant/chemokine system may make blocking a single receptor problematic as a therapeutic approach [24], various approaches to inhibit FPR1 signaling have nevertheless been considered for therapeutic development. For example, natural compounds have been evaluated for their ability to inhibit leukocyte chemotaxis, reactive oxygen species (ROS) production, and human neutrophil elastase (HNE) release by targeting *f*MLF-induced signaling cascades. Since excess NADPH-oxidase generated ROS, HNE, and other neutrophil-derived proteases can promote inflammation and numerous pathological conditions [25–27], this approach could have significant clinical benefit if effective and specific inhibitors were identified.

Of particular interest is the possibility that FPR1 antagonists that can transiently inhibit neutrophil responses to formylated peptides could be used as therapeutic agents in the treatment of inflammatory diseases. In efforts to discover new anti-inflammatory agents from natural sources, inhibitory activity of several hundred compounds derived from bacteria, higher plants, algae, and marine corals have been screened for their ability to inhibit *f*MLF-induced functional responses in neutrophils. These products include a wide range of compound classes from peptides to secondary metabolites, such as flavonoids, coumarins, quinones, naphtalenones, lignans, terpenoids (sesquiterpene lactones, diterpenes, triterpenes), steroids, alkaloids and other compounds. Some of these compounds blocked FPR1 directly, while others inhibited downstream *f*MLF-induced pathways.

In this review, we will summarize the outcome of previous screening efforts and reconsider these studies with a specific focus on FPR1 antagonists. For some of these previously reported natural compounds, we also conducted additional screening of analogs and similar compounds for their ability to antagonize FPR1 activation by *f*MLF in human neutrophils and FPR1-transfected cells. Because a high possibility exists that compounds capable of inhibiting *f*MLF signaling may target FPR1 as one of their molecular mechanisms of action, we also used pharmacophore modeling and molecular docking studies to predict how well various natural compounds fit the FPR1 antagonist pharmacophore and their potential for binding to FPR1.

2. Natural peptides and their derivatives as FPR1 antagonists

2.1. Cyclosporines and other cyclic peptides

The hydrophobic cyclic peptides, cyclosporine A (CsA) and H (CsH), are among most potent and receptor-specific FPR1 antagonists described so far [28,29]. Both cyclosporines were first isolated as undecapeptides from the fungus *Tolypocladium inflatum*. Although CsA and CsH dose-dependently displaced [^{3}H]-*f*MLF in FPR1-transfected rat basophilic leukemia (RBL) cells with IC_{50} values of 1.8 μM and 100 nM, respectively [30], they did not exhibit any obvious inhibitory effects on FPR2-mediated cellular functions [29,30]. Loor et al. [31] conducted comprehensive structure–activity relationship (SAR) analysis of sixty naturally occurring or biosynthetically produced cyclosporine analogs for their ability to inhibit *f*MLF-induced responses in differentiated HL-60 cells. Some of these cyclosporines, including naturally occurring FR901459 and SDZ 214-103, inhibited *f*MLF-induced *N*-acetyl-β-D-glucosaminidase release with nanomolar IC_{50} values [31]. Cyclosporine FR901459 was nearly 20-fold more potent than CsA but 4-fold less potent than CsH for inhibiting the FPR1-dependent response [32]. Extensive chemical modification of the cyclosporine scaffold led to the discovery of various non-immunosuppressive cyclophilin inhibitors for the treatment of hepatitis C infection and other diseases [33,34]. However, the effects of these synthetic cyclosporine analogs on FPR functions have not been reported.

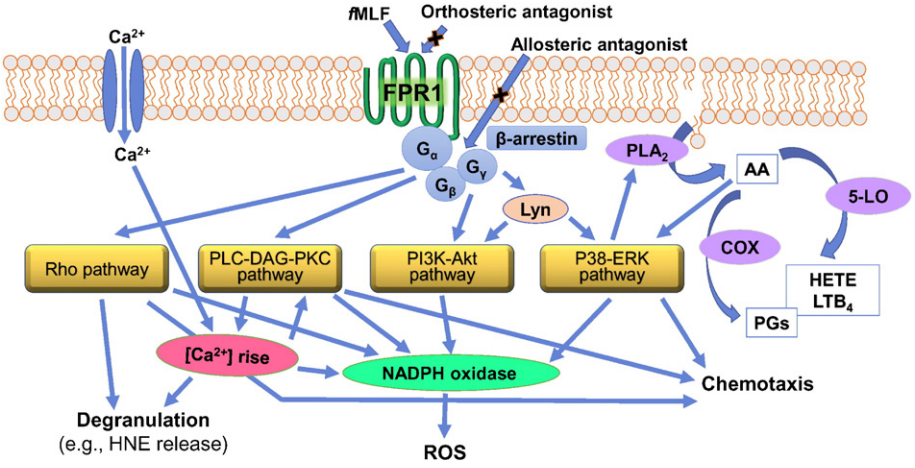

Fig. 1. Summary of *f*MLF-induced signaling events in human neutrophils. Upon *f*MLF binding, trimeric G proteins are uncoupled from formyl peptide receptor 1 (FPR1), and a series of signal transductions events ensue, resulting in neutrophil degranulation, including release of human neutrophil elastase (HNE), activation of the NADPH oxidase to produce reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\bullet}$), and chemotaxis. FPR1 activation can be blocked by either orthosteric or allosteric antagonists. The second messengers resulting from FPR1 activation regulate three main intracellular kinase pathways, including phosphatidylinositol-3-kinase (PI3K), protein kinase C (PKC), and mitogen-activated protein (MAP) kinases p38/extracellular signal related kinases 1 and 2 (ERK 1/2), leading to the array of functional responses listed. In addition, PKC-independent pathways can be activated through the Src-related tyrosine kinase, Lyn. Rho GTPase pathways also play key roles in regulating a variety of neutrophil functional responses. PKC-independent pathways can be activated through the Src-related tyrosine kinase, Lyn. Activation of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) and elevation in intracellular Ca^{2+} ($[Ca^{2+}]_i$) also results in the activation phospholipase A_2 (PLA_2), leading to arachidonic acid (AA) release. AA is metabolized by 5-lipoxygenase (5-LO) and cyclooxygenase (COX), and products of this metabolism [hydroxyeicosatetraenoic acid (HETE), leukotriene B_4 (LTB_4), prostaglandins (PGs), etc.] can act in an autocrine manner. As discussed in the text, various natural compounds can interfere with receptor binding and downstream intracellular signaling events.