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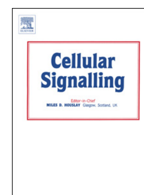

Apremilast is a selective PDE4 inhibitor with regulatory effects on innate immunity

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ABSTRACT

Apremilast, an oral small molecule inhibitor of phosphodiesterase 4 (PDE4), is in development for chronic inflammatory disorders, and has shown efficacy in psoriasis, psoriatic arthropathies, and Behçet's syndrome. In March 2014, the US Food and Drug Administration approved apremilast for the treatment of adult patients with active psoriatic arthritis. The properties of apremilast were evaluated to determine its specificity, effects on intracellular signaling, gene and protein expression, and in vivo pharmacology using models of innate and adaptive immunity. Apremilast inhibited PDE4 isoforms from all four sub-families (A1A, B1, B2, C1, and D2), with IC_{50} values in the range of 10 to 100 nM. Apremilast did not significantly inhibit other PDEs, kinases, enzymes, or receptors. While both apremilast and thalidomide share a phthalimide ring structure, apremilast lacks the glutarimide ring and thus fails to bind to cereblon, the target of thalidomide action. In monocytes and T cells, apremilast elevated intracellular cAMP and induced phosphorylation of the protein kinase A substrates CREB and activating transcription factor-1 while inhibiting NF-κB transcriptional activity, resulting in both up- and down-regulation of several genes induced via TLR4. Apremilast reduced interferon-α production by plasmacytoid dendritic cells and inhibited T-cell cytokine production, but had little effect on B-cell immunoglobulin secretion. In a transgenic T-cell and B-cell transfer murine model, apremilast (5 mg/kg/day p.o.) did not affect clonal expansion of either T or B cells and had little or no effect on their expression of activation markers. The effect of apremilast on innate immunity was tested in the ferret lung neutrophilia model, which allows monitoring of the known PDE4 inhibitor gastrointestinal side effects (nausea and vomiting). Apremilast significantly inhibited lung neutrophilia at 1 mg/kg, but did not induce significant emetic reflexes at doses <30 mg/kg. Overall, the pharmacological effects of apremilast are consistent with those of a targeted PDE4 inhibitor, with selective effects on innate immune responses and a wide therapeutic index compared to its gastrointestinal side effects.

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Abbreviations: ANOVA, analysis of variance; ATF-1, activating transcription factor-1; CCL-2, chemokine ligand 2; CCL-8, chemokine ligand 8; CCL-18, chemokine ligand 18; CCR-1, chemokine receptor 1; CRBN, cereblon; CRE, cAMP responsive element; CREB, cAMP responsive element binding protein; CXCL-5, epithelial-derived neutrophil activating protein 78; DCs, dendritic cells; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; ED_{50}, half-maximal effective drug concentration; ELISA, enzyme-linked immunosorbent assay; Epac, exchange proteins activated by cAMP; FP, fluorescence polarization; HEL, hen egg lysozyme; IC_{50}, half-maximal inhibitory concentration; Ig, immunoglobulin; IL, interleukin; IP-10, interferon-inducible protein 1; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MCP-2, monocyte chemoattractant protein 2; MHC, major histocompatibility complex; MIP-1αR, macrophage inflammatory protein 1-α receptor; MIP-4, macrophage inflammatory protein-4; MX1, myxovirus resistant 1; NF-κB, nuclear factor-kappa B; NK, natural killer; OVA, ovalbumin; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PDE4, phosphodiesterase 4; PKA, protein kinase A; PsA, psoriatic arthritis; SOCS-3, suppressor of cytokine signaling 3; TLR, toll-like receptor; TNF, tumor necrosis factor.

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1. Introduction

Inflammatory conditions, such as psoriasis and psoriatic arthritis (PsA), are related to a dysregulated immune system governed by a pro-inflammatory cytokine network [1–3]. The network of pro-inflammatory mediators that drive psoriasis and PsA are released by a variety of cell types, including innate or adaptive immune cells, and resident non-immune cells [1–3]. The cyclic nucleotides cAMP and cGMP are naturally occurring intracellular secondary messengers critical to translating extracellular stimuli into intracellular signals that control gene expression, allowing the cell to interact with its environment and regulate broader physiological processes, including those involved in inflammation [4]. In the presence of inflammatory extracellular signals, G-protein-coupled receptors bind with a variety of ligands, such as leukotrienes, prostaglandins, chemokines, and histamine, and activate adenylyl cyclase, which promotes increased production of cAMP [5]. cAMP interacts with effector proteins such as protein kinase A (PKA) and exchange proteins activated by cAMP (Epac) to elicit changes in gene expression [6]. PKA activation results in phosphorylation of the cAMP-responsive binding element family of transcription factors, including cAMP responsive element binding protein (CREB) and activating transcription factor-1 (ATF-1), while inhibiting activity of other promoters such as nuclear factor kappa B (NF-κB) [3,7,8]. Such effects on CREB, ATF-1, and NF-κB cause decreased mRNA expression of cytokines and other inflammatory mediators as well as increased expression of anti-inflammatory signals [5,8]. In this way, cAMP signaling helps to maintain immune homeostasis by modulating the production of pro-inflammatory and anti-inflammatory mediators [5]. When intracellular cAMP concentrations are high, inflammatory signaling is dampened; likewise, when cAMP levels are depleted, expression of inflammatory mediators increases. By modulating the levels of inflammatory and anti-inflammatory mediators expressed and released by immune cells, cAMP is one component in a cascade that determines recruitment of immune responses both in the local milieu and throughout the body.

Intracellular levels of cAMP are tightly controlled by adenylyl cyclase, which promotes cAMP formation, and by cyclic nucleotide phosphodiesterases (PDEs), which are the only means of degrading cAMP, via enzymatic hydrolysis. There are 11 distinct families of cAMP and/or cGMP-selective PDEs expressed in mammalian species (PDE1–11), each containing a conserved catalytic domain in the carboxy-terminal portion of the enzyme, plus amino-terminal subdomains that are important for subcellular localization, and for interactions with signaling molecules and molecular scaffolds [9]. While certain PDEs specifically hydrolyze cAMP (PDE4, PDE7, and PDE8), or specifically hydrolyze cGMP (PDE5, PDE6, and PDE9), others hydrolyze both cAMP and cGMP (PDE1, PDE2, PDE3, PDE10, and PDE11) [9]. In most mammalian cells, PDE3 and PDE4 predominantly hydrolyze cAMP [9]. Unlike PDE3, PDE4 is cAMP-specific and the dominant PDE in inflammatory cells [3,10]. PDE4 is also expressed in structural cell types involved in psoriasis, such as keratinocytes, vascular endothelium, and synovium [11]. The PDE4 isoenzyme family is encoded by four genes (PDE4A, PDE4B, PDE4C, and PDE4D) and consists of more than 20 distinct isoforms, each with a unique N-terminal region, created by mRNA splicing and different promoters [4,12]. PDE4 isoforms are categorized as long, short, or super short depending on the presence and number of upstream conserved regions, highly conserved domains located between the catalytic domain and the N-terminal region; dead-short isoforms are those containing no upstream conserved regions and a truncated, nonfunctional catalytic domain [13]. In line with the structural diversity of the PDE4 family, the unique N-terminal region of each PDE4 isoform allows each to be sequestered by specific protein partners within sub-regions of the cell [12]. PDE4 inhibition elevates intracellular cAMP levels, which results in down-regulation of the inflammatory responses by reducing the expression of tumor necrosis factor (TNF)-α, interleukin (IL)-23, and other pro-inflammatory cytokines, while increasing anti-inflammatory cytokines, such as IL-10 [3,14]. Therefore PDE4 is of interest as a therapeutic target in the treatment of chronic inflammatory conditions [14,15]. Currently marketed PDE4 inhibitors include apremilast (Otezla®, Celgene Corporation, Summit, New Jersey) [16], approved in the United States for the treatment of adult patients with active psoriatic arthritis, and roflumilast (Daliresp®, Forest Pharmaceuticals, St. Louis, Missouri) [17] for the treatment of chronic obstructive pulmonary disorder.

Apremilast is an oral small molecule inhibitor of PDE4 [11,15,18] which has been shown to be effective and well tolerated in clinical trials in psoriasis (phase III), PsA (phase III), and Behçet's disease (phase II). Targeted inhibition of PDE4 results in partial inhibition of pro-inflammatory mediator production, such as TNF-α, interferon-γ, and IL-23, and increases in anti-inflammatory mediator production, such as IL-10 [3,15,19], which in turn results in reduced infiltration of immune cells and changes in resident cells of the skin and joints [11,15,19,20]. In vitro, apremilast significantly reduced expression of TNF-α, IL-7 and the matrix metalloproteinases MMP1, MMP3, MMP13, and MMP14 by synoviocytes derived from patients with rheumatoid arthritis [19,21,22]. In other cell culture models, apremilast inhibited the differentiation of osteoclasts, as well as their bone-resorbing activity, and reduced the production of RANKL by osteoblasts [23]. In patients with severe plaque psoriasis, apremilast reduced infiltration of myeloid dendritic cells (DCs) into the dermis and epidermis and inducible nitric oxide synthase mRNA expression; epidermal thickness was reduced by approximately 20% over 29 days [20]. A subsequent study in recalcitrant plaque psoriasis demonstrated that apremilast reduced epidermal and dermal infiltration of myeloid DCs, T cells, and natural killer (NK) cells, and inhibited the expression of genes in the Th1, Th17, and Th22 pathways in the psoriatic skin lesions, including IL-12/IL-23p40, IL-23p19, IL-17A, and IL-22 [24]. Phase II and phase III studies have demonstrated the clinical efficacy of apremilast in the treatment of patients with active PsA and moderate to severe plaque psoriasis, and phase II studies have demonstrated the efficacy of apremilast for patients with Behçet's disease [25–33].

The current analyses studied the pharmacodynamic properties of apremilast, with three specific aims: 1) ascertain the selectivity of apremilast by determining whether it binds to targets other than PDE4 in the cell; 2) define which signaling pathways downstream of PDE4 are modulated by apremilast; and 3) identify the repertoire of immune cells affected by the drug. Our data show that apremilast has no identified binding targets other than PDE4 and mediates its effects in monocytes and T cells via PKA and NF-κB pathways. Apremilast modulates gene expression in monocytes, reduces interferon-α production induced by TLR9 signaling in plasmacytoid dendritic cells, and inhibits cytokine production by T cells, but has little effect on immunoglobulin secretion by B cells in vitro. To assess its impact on the adaptive immune response, apremilast was tested in an antigen-specific transgenic mouse model of T- and B-cell clonal expansion, activation marker expression, and immunoglobulin production. Using the ferret as both a model of an innate inflammatory response, and for the gastrointestinal side effects of PDE4 inhibition, a therapeutic index was measured in vivo.

2. Material and methods

2.1. Materials

Celgene Corporation (Summit, New Jersey) synthesized apremilast (CC-10004 or [S]-N-[2-[1-3-ethoxy-4-methoxyphenyl]-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl] acetamide) and other PDE4 inhibitors, as well as thalidomide, lenalidomide (CC-5013), and pomalidomide (CC-4047). Forskolin was obtained from Sigma (St. Louis, Missouri) and dimethyl sulfoxide (DMSO), used to generate stock solutions, was obtained from Research Organics (Cleveland, Ohio).

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