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Therapeutic Advances in Musculoskeletal Disease 2010 2: 271 originally published online 16 August 2010
DOI: 10.1177/1759720X10381432

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What is This?
Apremilast: a novel PDE4 inhibitor in the treatment of autoimmune and inflammatory diseases

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Abstract: Phosphodiesterase 4 (PDE4) is a key enzyme in the degradation of cyclic adenosine monophosphate and is centrally involved in the cytokine production of inflammatory cells, angiogenesis, and the functional properties of other cell types such as keratinocytes. In this review article, apremilast, a novel small molecule inhibitor of PDE4, is introduced. Apremilast has profound anti-inflammatory properties in animal models of inflammatory disease, as well as human chronic inflammatory diseases such as psoriasis and psoriatic arthritis. Apremilast blocks the synthesis of several pro-inflammatory cytokines and chemokines, such as tumor necrosis factor alpha, interleukin 23, CXCL9, and CXCL10 in multiple cell types. In contrast to the biologics, which neutralize pro-inflammatory mediators at the protein level, apremilast modulates production of these mediators at the level of mRNA expression. Apremilast also interferes with the production of leukotriene B4, inducible nitric oxide synthase, and matrix metalloproteinase and reduces complex inflammatory processes, such as dendritic cell infiltration, epidermal skin thickening, and joint destruction. As this novel PDE4 inhibitor interferes with several key processes of inflammation, it may emerge as a promising new drug for the treatment of chronic inflammatory diseases such as those of the skin and the joints.

Keywords: apremilast, cyclic adenosine monophosphate, phosphodiesterase 4, psoriasis, psoriatic arthritis

Introduction
Cyclic adenosine monophosphate (cAMP) is a second messenger that plays a key role in the regulation of many biologic responses in humans, including inflammation, apoptosis, and lipid metabolism. This regulation is a result of the cAMP and protein kinase A (PKA) pathway, a common and versatile signaling mechanism in eukaryotic cells that is involved in the regulation of various cellular functions [Tasken and Aandahl, 2004]. In this molecular pathway, cAMP is the major second messenger responsible for intracellular signal transduction by means of G-protein coupled receptors, such as histamine, α- and β-adrenergic receptors, prostaglandin, and N-methyl-D-aspartic acid. Activation of these receptors by their respective ligands induces a change in the cAMP concentration within the cell that can vary in duration, amplitude, and extension. Adenyl cyclases that form cAMP, as well as phosphodiesterases that degrade cAMP, can shape these changes [Tasken and Aandahl, 2004].

Phosphodiesterase 4
Phosphodiesterases (PDEs) are the only enzymes that hydrolyze and degrade cAMP [Conti and Beavo, 2007]. PDE4 is a cAMP phosphodiesterase widely expressed in hematopoietic cells (e.g. myeloid, lymphoid), nonhematopoietic cells (e.g. smooth muscle, keratinocyte, endothelial), and sensory/memory neurons [Houslay and Adams, 2003]. The four PDE4 genes (A, B, C, and D) exhibit distinct target and regulatory properties [Houslay et al., 2007]. Each of these genes can produce multiple protein products due to mRNA splice variants, resulting in approximately 19 different PDE4 proteins that fall into either short or long isoform categories. Long isoforms are differentiated from short isoforms by an
These UCR sequences play a critical role in the regulation of PDE4 through the phosphorylation of PKA and extracellular signal-regulated kinase (ERK). For example, the major PDE4 isoforms expressed in leukocytes are PDE4 B2 (short isoform) and PDE4 D3 and D5 (long isoforms) [Houslay et al. 2005]. Long PDE4 D isoforms predominate in monocytes, whereas short PDE4 B isoforms predominate in macrophages [Houslay et al. 2005]. The catalytic activity of PDE4 B2 is activated by ERK phosphorylation, whereas the catalytic function of the D3 and D5 variants is inhibited by ERK activation [Shepherd et al. 2004]. Thus, the UCR modules can determine the functional outcome of ERK phosphorylation. This means that the pro-inflammatory mediators of monocytes trigger an overall decrease in PDE4 activity, whereas the pro-inflammatory mediators of macrophages trigger an overall increase in PDE4 activity. Thus, the relative enzymatic activities of short and long isoforms may differ upon cell activation.

The UCR also provides an interface for interaction with other proteins, such as myomegalin (a scaffolding protein), the immunophilin XAP2 [Bolger et al. 2003], and phosphatidic acid [Richter and Conti, 2004]. The beta-arrestin interaction site of PDE4 is important for association with the T-cell costimulatory molecule CD28. Interacting proteins on the N-terminus are specific to individual PDE4 isoforms. For instance, the N-terminus of PDE4 A5 interacts with XAP2, a protein involved in the aryl hydrocarbon receptor pathway, whereas the N-terminus of PDE4-D5 engages RACK1, a protein involved in the protein kinase C pathway [Yarwood et al. 1999].

In inflammatory cells, smooth muscle cells, endothelial cells, and keratinocytes, PDE4 enzymes play an important role in degrading cAMP [Houslay et al. 2007, 2005]. PDE4 regulates leukocyte responses, which includes the pro-inflammatory actions of leukocytes (e.g. monocytes, T cells, neutrophils), airway/vascular smooth muscle constriction, and neurotransmitter signaling through adenylyl cyclase-linked G-protein receptors (e.g. N-methyl-D-aspartate). Monocytes and macrophages are the main producers of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α), whose levels are decreased upon inhibition of PDE4 [Schafer et al. 2010; Jimenez et al. 2001; Seldon et al. 1995]. Interleukin 12 (IL-12) production in macrophages, which is important for the differentiation of T-helper 1 cells, is also regulated by PDE4 [Liu et al. 2000].

The effect of PDE4 inhibition on signal transduction and cytokine gene transcription in monocytes and macrophages is depicted in Figure 1.

**Figure 1.** Mechanism of action of PDE4 inhibition in monocytes.
In human peripheral blood monocytes, TNF-α production induced by the bacterial endotoxin lipopolysaccharide (LPS) is inhibited by a variety of agents that activate the PKA pathway, including β2-adrenoceptor agonists, 8-bromo-cAMP (which also activates the exchange proteins activated by cAMP [EPACs], or guanine nucleotide exchange factors), cholera toxin, and prostaglandin E2, as well as various PDE4 inhibitors including rolipram, denbufylline, Ro 20-1724, and benafentrine [Seldon et al. 1995]. In both monocytes and endothelial cells, cAMP elevation caused by the adenylate cyclase activator forskolin or treatment with dibutyryl cAMP inhibits nuclear factor-κB (NF-κB)-dependent gene transcription [Ollivier et al. 1996]. This effect is not mediated on nuclear translocation or phosphorylation of the NF-κB subunits p65, p50, or c-Rel, but rather by direct inhibition of NF-κB transcriptional activity [Ollivier et al. 1996]. In monocytes and macrophages chronically treated with ethanol, there is an increase in PDE4B expression, reduction of cellular cAMP levels, and enhancement of NF-κB activation, all leading to increased TNF-α production upon LPS stimulation [Gobejishvili et al. 2008]. Even in these ethanol-primed cells, the PDE4 inhibitor rolipram reduces TNF-α production at the mRNA level, also by a mechanism involving decreased NF-κB transcriptional activity [Gobejishvili et al. 2008]. This mechanism was confirmed and further defined more recently using the PDE4 inhibitor roflumilast, which elevated intracellular cAMP levels, inhibited nitric oxide production, and reduced expression of inducible nitric oxide synthase (iNOS) and TNF-α in LPS-stimulated mouse macrophages [Kwak et al. 2005]. These effects were associated with a decrease in NF-κB p65 DNA binding, which was attributed to a decrease in phosphorylation of the inhibitor of κBα (IκBα) [Kwak et al. 2005]. Therefore, inhibition of PDE4 leads to inhibition of TNF-α gene expression by a cAMP, PKA, and NF-κB-dependent mechanism. In contrast to TNF-α, expression of the anti-inflammatory cytokine IL-10 is enhanced by PDE4 inhibitors in a PKA-dependent manner [Eigler et al. 1998]. The mechanism of this enhancement involves multiple cAMP responsive elements (CREs) within the IL-10 promoter and enhancer, which recruit the CRE binding proteins CREB and ATF-1, substrates of PKA [Platzer et al. 1999]. Although IL-10 can exert its own inhibitory effect on TNF-α production in monocytes, these two opposite effects of PDE4 inhibitors appear to be independent, since neutralizing anti-IL-10 antibodies do not abrogate the inhibitory effect of PDE4 inhibition on TNF-α [Seldon et al. 1998]. As these examples of TNF-α and IL-10 regulation by the cAMP/PKA axis illustrate, the effects of PDE4 inhibition on gene expression can be either negative or positive, depending upon the presence of NF-κB or CRE elements within the gene promoter.

In T cells, PDE4 plays a critical role in signal transduction by virtue of its association with the CD28 surface receptor [Bjørgo and Taskén, 2010]. A summary of the mechanism of PDE4 involvement in T-cell activation is depicted in Figure 2. The production of TNF-α, IL-2, IL-4, and IL-5 and the proliferation of T lymphocytes are all dependent upon PDE4 activity [Essayan et al. 1997, 1994]. It has been shown that inhibition of PKA increases T-cell receptor-induced immune responses, and that inhibition of PDE4 blunts T-cell cytokine production [Abrahamsen et al. 2004]. Overexpression of PDE4 results in augmented T-cell receptor/CD28-stimulated cytokine production. PDE4 is recruited to the cell surface upon cross-ligation of CD28 and T-cell receptor, where it can reduce local cAMP levels, thereby eliminating the PKA/Csk/Lck inhibitory pathway [Borska et al. 2007]. This allows Lck to be fully activated, which leads to cytokine production by T cells. When PDE4 activity is inhibited in T cells, PKA becomes activated and the Csk/Lck inhibitory axis is restored. Downstream, this results in the inhibition of NF-κB and nuclear factor of activated T cells [Jimenez et al. 2001]. PDE4 also regulates the proliferative response of T cells, as demonstrated by the ability of rolipram to suppress the antigen-induced proliferation of lymphocytes [Essayan et al. 1997].

With respect to neutrophils, PDE4 has been shown to be involved in the production of IL-8, leukotriene B4, and superoxide anions facilitating degranulation and chemotaxis of neutrophils. Moreover, PDE4 mediates the adhesion of neutrophils by inducing the expression of the β2-integrin Mac-1, which mediates adhesion to vascular wall endothelium [Houslay et al. 2005; Jones et al. 2005]. Eosinophil functions, such as degranulation and chemotaxis, are regulated by PDE4 [Alves et al. 1996]. With respect to the vascular endothelium, PDE4 inhibitors have an anti-angiogenic effect by inhibiting TNF-α induced E-selectin expression on endothelial
cells, as well as blocking vascular endothelial growth factor–induced endothelial cell migration.

**Apremilast: mode of action**

Apremilast (\((\text{S})-N\{-2-[1-(3-\text{Ethoxy}-4-\text{methoxy-phenyl})-2-\text{methanesulfonylethyl}]\,1,3\text{-dioxo-2,3-dihydro-1H-isoindol-4-yl}\}\text{acetamide}\)), also known as CC-10004 (CAS registry number 608141-41-9), has a molecular weight of 460.5. Apremilast binds to the catalytic site of the PDE4 enzyme, thereby blocking cAMP degradation. When initially screened for PDE4 inhibition, apremilast was found to exhibit an IC\(_{50}\) of approximately 0.074 \(\text{mM}\) [Man et al. 2009]. The \(K_i\) value (affinity constant) of apremilast for PDE4 is 68 \(\text{nM}\). Apremilast is a partial competitive inhibitor of PDE4 based on Lineweaver-Burk analysis [Schafer et al. 2010]. The compound did not demonstrate any marked PDE4 subfamily selectivity in the cAMP assays for PDE4 A4, B2, C2, and D3 with similar potencies at IC\(_{50}\)s ranging from 20 to 50 nM, thus not representing a PDE4 subtype-selective inhibitor [Schafer et al. 2010]. In comparison, cilomilast is 10-fold more selective for PDE4D [Giembycz, 2001]. The PDE4D isozyme has been associated with the behavioral correlate of emesis in mice [Robichaud et al. 2002]. The lack of PDE4D selectivity of apremilast may in part explain its improved therapeutic index compared with cilomilast in nonclinical models [Schafer, 2005]. Also apremilast, unlike rolipram, another PDE4 inhibitor, does not induce central nervous system effects, such as lethargy and fatigue [McCann et al. 2010].

Upon LPS challenge, apremilast inhibits the production of TNF-\(\alpha\) (IC\(_{50}\) = 0.11 \(\text{mM}\)), IFN-\(\gamma\) (IC\(_{50}\) = 0.013 \(\text{mM}\)), and IL-12p70 (IC\(_{50}\) = 0.12 \(\text{mM}\)), as well as the chemokines CXCL9 (MIG), CXCL10 (IP-10), and CCL4 (MIP1\(\alpha\)) from human peripheral blood mononuclear cells [Schafer et al. 2010]. One of the important features of apremilast activity in cells is that it retains its cellular potency in the whole-blood setting. For example, apremilast inhibits TNF-\(\alpha\) production in whole blood (IC\(_{50}\) = 0.11 \(\text{mM}\)) with potency similar to that in isolated cells. Clinically relevant concentrations of apremilast, based on the maximal plasma concentrations observed after the 20 mg dose, are in the range of 450 \(\text{nM}\) (207.07 ng/ml) [Gottlieb et al. 2008]. With respect to human T lymphocytes, apremilast inhibits the synthesis of T-cell-derived cytokines in vitro. This includes the expression of IL-2 (IC\(_{50}\) = 0.29 \(\text{mM}\) and IFN-\(\gamma\) (IC\(_{50}\) = 0.046 \(\text{mM}\)) [Schafer et al. 2010]. With respect to neutrophils, apremilast inhibits zymosan-induced production of IL-8, which is a chemokine required for

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**Figure 2.** Mechanism of action of PDE4 inhibition in T cells.
neutrophil chemotaxis to inflamed tissues. Inhibition of IL-8 expression was associated with a 75% inhibition of neutrophil chemokinesis at a concentration of apremilast 10 μM. In addition, apremilast inhibited N-formyl-Met-Leu-Phe-induced CD18 and CD11b expression leading to impaired adhesion of polymorphonuclear cells (PMN) to human umbilical vein endothelial cells. The adherence of neutrophils to the vascular wall endothelium is mediated by inducible expression of the β2-integrin Mac-1. Mac-1 consists of a CD18/CD11b heterodimer and a lymphocyte function-associated antigen 1. Also, leukotriene B4, a product of arachidonic acid metabolism with chemoattracting properties for neutrophils, is inhibited by apremilast.

In lamina propria mononuclear cells isolated from gut mucosa, apremilast inhibited pokeweed mitogen-stimulated TNF-α production. Furthermore, in lamina propria mononuclear cells isolated from Crohn’s disease or ulcerative colitis patients, apremilast inhibited the spontaneous release of MMP-3, one of the metalloproteinases that mediate the mucosal damage triggered by TNF-α production in inflammatory bowel disease [Gordon et al. 2009]. This illustrates the capability of PDE4 inhibition to interfere with a pro-inflammatory pathway at more than one point along the cascade toward tissue damage. In addition, these data highlight the potential of apremilast to treat inflammatory diseases of the gastrointestinal tract.

With respect to nonhematopoietic cells, an inhibitory effect of apremilast has been shown on TNF-α production by synovial membrane cultures derived from rheumatoid arthritis patients. Apremilast inhibits TNF-α production from rheumatoid synovial membrane cultures by 46% at 100 nM [McCann et al. 2010]. Moreover, apremilast also inhibits TNF-α production from UV-treated keratinocytes, which is important for inflammatory skin diseases such as psoriasis. In contrast, apremilast did not have any significant effect on normal keratinocyte proliferation or viability at concentrations of 0.0001–10 μM, suggesting that it would not have an unwanted thinning effect on skin.

**Apremilast in psoriasis**

Psoriasis is a chronic inflammatory dermatosis characterized by the proliferation of hyperproliferative epidermal keratinocytes. The cellular immune system is thought to trigger this keratinocyte response, with monocytes, dendritic cells, neutrophils, and T cells being implicated in the pathogenesis. PDE4 is present in all inflammatory cells identified to be relevant in psoriasis and appears to be involved in several pathophysiological processes of psoriasis such as (1) the production of TNF-α, IL-12, and IL-23 by monocytes/macrophages, (2) the synthesis of IL-2, IFN-γ, and IL-5 production by T lymphocytes, and (3) the expression of TNF-α and IFN-α by plasmacytoid dendritic cells. Moreover, chemoattraction via regulation of IL-8 and IP-10 expression, and TNF-α production by keratinocytes, are both influenced by PDE4. Preclinical *in vivo* testing of apremilast has been performed in natural killer cell-driven models of psoriasis using human skin xenotransplanted onto immunodeficient SCID mice, followed by challenge with human natural killer cells [Papp et al. 2008; Bos et al. 2005]. Injection of natural killer cells from psoriatic donors into nonlesional psoriatic skin results in a classic psoriasis histology. In this model, apremilast (5 mg/kg orally, divided into two daily doses) significantly reduced keratinocyte proliferation, skin thickness, and the general histopathologic appearance of psoriasiform features [Schafer et al. 2010]. Moreover, expression of TNF-α, ICAM-1, and HLA-DR on the skin grafts was qualitatively reduced upon apremilast treatment.

In a small, open-label, phase II pilot study in patients with severe psoriasis treated with apremilast 20 mg once daily for 29 days, skin biopsies were obtained and analyzed for epidermal thickening, histological appearance, specific leukocyte subsets, and inflammatory gene expression. Also, whole blood was drawn for *ex vivo* LPS stimulation and TNF-α measurement. In this study, 8 of the evaluable 15 patients met the primary endpoint of achieving a ≥20% reduction in epidermal thickness. Overall, the patients showed a mean decrease of 20.5% in epidermal thickness. This response was associated with a 28.8% and 42.6% decrease in T cells in the dermis and epidermis, respectively, and by an 18.5% and 40.2% decrease in CD11c⁺ myeloid dendritic cells in the dermis and epidermis, respectively. Within the skin biopsies, there was a significant reduction in the mRNA levels of inducible nitric oxide synthetase after 1 month of treatment with apremilast [Gottlieb et al. 2008]. Also in this study, apremilast had an inhibitory effect on *ex vivo* whole-blood LPS-stimulated TNF-α production.
2 hours after the first dose, in 11 of the patients. Therefore, in this short study using a low dose of apremilast, meaningful changes in both skin and blood biomarkers were observed. Subsequently, a randomized, placebo-controlled phase II study was performed in 260 patients with moderate to severe psoriasis using two doses of apremilast (20 mg once daily and 20 mg twice daily) [Papp et al. 2008]. In this study, a 75% reduction in the Psoriasis Area and Severity Index score was observed in 24% of patients receiving apremilast 20 mg twice daily compared with placebo (10%; \( p = 0.023 \)), whereas the clinical response to the lower, once-daily 20 mg dose was not significantly different from placebo. Moreover, a 50% improvement in the Psoriasis Area and Severity Index score was observed in a significantly greater percentage (57%) of apremilast-treated patients compared with 23% of the patients receiving placebo (\( p < 0.001 \)). Doses of apremilast 20 mg twice daily led to plasma concentrations between 129 ng/ml \( (C_{\text{min}}) \) and 389 ng/ml \( (C_{\text{max}}) \). With respect to safety, apremilast was generally well tolerated. Diarrhea (5–10%) and nausea (3–5%) were more commonly observed in patients treated with the two doses of apremilast than with placebo. Most cases were mild to moderate in severity. Gastrointestinal symptoms are in fact not unexpected and considered as a class effect of PDE4 inhibitors. Owing to the lack of highly specific inhibition of the PDE4D isoform, however, these effects were mild.

In psoriasis, the pharmacokinetic profile of apremilast has also been characterized. Patients receiving 20 mg apremilast once daily showed a mean steady-state maximal concentration \( (C_{\text{max}}) \) of 207.07 ng/ml and the area under the curve (AUC) was 1799 ng·h/ml. The median time oral administration of apremilast reached a maximal concentration \( (T_{\text{max}}) \) was 2 hours, the mean half-life of the drug was 8.2 h. With respect to excretion of the drug, the mean clearance \( (\text{CL}/\text{F}) \) was 10.41 l/h, and mean volume of distribution \( (V_{Z}/F) \) was 1281 [Gottlieb et al. 2008].

**Apremilast in arthritis**

Apart from psoriasis, there is also a rationale for using apremilast to control the inflammatory disease process of arthritis. Arthritis is characterized by a massively enhanced influx of immune cells, in particular monocytes/macrophages, neutrophils, and lymphocytes into joints. In addition, there is a strong mesenchymal response in arthritis, which manifests as synovial hyperplasia based on proliferation of synovial fibroblast-like cells. Both immune cells and synovial fibroblasts contribute to cytokine production in the inflamed synovium, leading to a perpetuation of the inflammatory response, as well as to cartilage and bone resorption caused by activation of osteoclasts. Apremilast was tested in animal models of arthritis and human inflammatory arthritis. In collagen-induced arthritis of DBA1 mice, apremilast showed a reduction of clinical and histopathologic signs of arthritis at doses of 5 and 25 mg/kg administered by daily intraperitoneal injections [McCann et al. 2010]. *Ex vivo* experiments showed that apremilast inhibited T-cell proliferation, IFN-\( \gamma \) production, and TNF-\( \alpha \) production in stimulated lymph node cells from collagen-immunized mice at concentrations as low as 0.1\( \mu \)M. Similar results were obtained in experimental arthritis induced by monoclonal antibodies against type II collagen: apremilast, at an oral dose of 25 mg/kg, significantly blocked synovial inflammation, cartilage damage, and bone erosion in BALB/c mice. At the lower dose of 5 mg/kg, significant inhibition of paw swelling was observed by the end of the study [McCann et al. 2010].

These preliminary studies eventually led to further clinical development and a multicenter, phase II, randomized, double-blind, placebo-controlled study that examined the efficacy and safety of apremilast in patients with psoriatic arthritis. Apremilast demonstrated statistically significant efficacy with an acceptable safety and tolerability profile [Schett et al. 2009].

**Summary**

The available phase 2 data support the clinical efficacy and safety of apremilast in the treatment of patients with psoriasis and psoriatic arthritis and warrant further development in phase 3. In addition, these results suggest that apremilast may be effective as an orally active agent for the treatment of other chronic inflammatory diseases. Consequently, studies are planned or being conducted in other indications, such as ankylosing spondylitis, rheumatoid arthritis, and Behçet disease to further extend the clinical spectrum of this drug.

**Funding**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
Conflict of interest statement
PS, VS, and RS are employees of Celgene Corporation, and are holders of company stock and stock options.

References


