Anti-Inflammatory Potential of the Selective Phosphodiesterase 4 Inhibitor \(N\)-(3,5-Dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic Acid Amide (AWD 12-281), in Human Cell Preparations

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ABSTRACT
AWD 12-281 is a potent (IC_{50} = 9.7 nM) and highly selective inhibitor of the phosphodiesterase 4 (PDE4) isoenzyme with low affinity to the high-affinity rolipram-binding site. The compound was optimized for topical treatment of asthma, chronic obstructive pulmonary disease (COPD), and allergic rhinitis. The aim of the present study was to assess the effect of AWD 12-281 in human inflammatory cells. Peripheral blood mononuclear cells (PBMCs), diluted whole blood, and human nasal polyp cells derived from surgically resected nasal polyps with polyposis comprise sources of target tissue cells that can be used to predict anti-inflammatory effects in patients. AWD 12-281 was capable of suppressing the production of cytokines in stimulated PBMCs: interleukin-2 (IL-2, phytohemagglutinin stimulation), IL-5 (concanavalin A stimulation), IL-5 and IL-4 (anti-CD3/anti-CD28 co-stimulation), and lipopolysaccharide-stimulated release of tumor necrosis factor \(\alpha\) (TNF\(\alpha\)). The corresponding values for half-maximum inhibition, EC_{50}, for AWD 12-281 were within a narrow range (46–121 nM). Comparing the effect of AWD 12-281 with roflumilast, cilomilast (SB 207499), rolipram (RPR-73401), and 1-(3-nitrophenyl)-3-(4-pyridylmethyl)pyrido[2,3-d]pyrimidin-2,4(1H,3H)-dione (RS-25344-000), it could be shown that the PDE4 inhibitory activity was closely correlated with inhibitory potential as measured by the above-described assays. AWD 12-281 was also shown to suppress TNF\(\alpha\) release in dispersed nasal polyps (EC_{50} = 111 nM) and in diluted whole blood (EC_{50} = 934 nM). The reduced activity in human blood may be related to high plasma protein binding. Currently, phase II clinical studies are under way to evaluate the therapeutic potential of AWD 12-281 in asthma, COPD, and allergic rhinitis.

To date, 11 families of phosphodiesterases (PDEs) have been identified. These enzymes are critically involved in the modulation of signal transduction and act by degradation of cyclic nucleotides (cAMP and/or cGMP). Among the PDEs, PDE4, PDE7, and PDE8 show specificity for cAMP (Conti and Yin, 1999; Soderling and Beavo, 2000). In the search for an anti-inflammatory treatment alternative to corticosteroids, selective PDE4 inhibitors have received considerable attention, because the PDE4 isoenzyme is the major cAMP-metabolizing enzyme in immune and inflammatory cells (Torphy et al., 1992; Schmidt et al., 1999). Selective inhibition of PDE4 has anti-inflammatory effects in patients with inflammatory diseases such as asthma or chronic obstructive pulmonary disease (COPD) (Compton et al., 2001; Leichtl et al., 2002; Barnes, 2003).

Currently, two compounds, i.e., roflumilast and cilomilast, are in clinical development (phase III) for the treatment of COPD and, in the case of roflumilast, for asthma as well. PDE4 inhibitors are potentially superior to corticosteroids in clinical use, because they not only induce anti-inflammatory effects but also influence diverse other cell types involved in these diseases; these cell types include respiratory epithelial cells, smooth muscle cells and submucosal glands (Wright et al., 1998; Le Jeune et al., 2002; Braunstahl et al., 2003; Wenzel et al., 2003). Furthermore, there are data indicating that PDE4 inhibitors of the new generation lack the side effects frequently associated with corticosteroid therapy, including inter alia adverse effects on the pituitary-hypophyseal axis and on bone density (Cave et al., 1999; Burnouf and
Pruniaux, 2002; Leonard and Sur, 2002; Tattersfield et al., 2002). However, further side effects, such as nausea, vomiting, increased gastric acid secretion, and headache, have so far limited the therapeutic use of PDE4 inhibitors (Montana and Hazel, 2002).

AWD 12-281 is a structurally new and highly selective PDE4 inhibitor (Kuss et al., 2002; Marx et al., 2002; Kuss et al., 2003). This compound was shown to have a better safety profile than other PDE4 inhibitors in clinical development. Furthermore, it was designed for topical treatment. It has a low oral bioavailability and a low solubility, and it exerts strong and long-lasting pharmacological effects after intratracheal administration in various animal models, indicating persistence in lung tissue. High plasma-protein binding and efficient hepatic metabolism by glucuronidation are additional factors contributing to low systemic exposure after intratracheal dosing. These factors contribute to a unique difference between emetic and anti-inflammatory dose levels, e.g., by a factor of >100 in ferrets (Kuss et al., 2003).

The aim of the present study was to assess the effect of AWD 12-281 in human inflammatory cells. The human cell preparations, PBMCs, diluted whole blood, and human nasal polyp cells derived from surgically resected nasal polyps in patients with polyposis comprise sources of target tissue cells that can be used to predict anti-inflammatory effects in patients. We selected the release of tumor necrosis factor alpha (TNFα) as marker for inflammatory processes in all cell preparations tested. In addition, the release of the cytokines IL-2, IL-4, and IL-5 was determined in PBMCs. TNFα is released by activated monocytes, macrophages, and lymphocytes, and the activity of TNFα is mediated through the binding to membrane-bound TNF receptors.

IL-4 is critically important for the development of allergic inflammation. It induces the IgE production of B lymphocytes (Coffman et al., 1986) and causes activation of the mast cells. IL-4 is essential to allergic inflammation because it is able to drive the differentiation of Th0 lymphocytes into Th2 lymphocytes (Hsieh et al., 1992). IL-5 plays a key role in the development and activation of eosinophils and therefore also in eosinophilic inflammation (Leckie and Walker, 2001). Th2 lymphocytes are the principal source of IL-5 (Ying et al., 1995). IL-2 is a cytokine derived from Th1 lymphocytes and is involved in the growth and differentiation of T cells and also in eosinophilic inflammation in vivo. Some of these data have already been published in abstract form (Heer et al., 1999, 2000; Kuesters et al., 1999).

Materials and Methods

Reagents and Inhibitors. The following reagents were used: fetal calf serum (FCS), Dulbecco’s phosphate-buffered saline (PBS), Hank’s balanced salt solution, trypan blue solution, penicillin/streptomycin, Biocoll (Biochrom AG Seromed, Berlin, Germany), human IgE, and anti-human IgE (Calbiochem, San Diego, CA); RPMI 1640 medium, PBS (In Vitrogen, Karlsruhe, Germany), Ficoll-Paque (Pharmacia AB, Uppsala, Sweden), [3H]AMP and SPA-beads (Amersham Biosciences Inc., Freiburg, Germany), LPS (Sigma Chemie, Deisenhofen, Germany), and anti-CD28 and IL-2, -4, -5, TNFα antibody pairs (BD Pharmingen, Erlangen, Germany), IL-4, -5, TNFα. Kits with precoated plates were from Coulter Immunotech Diagnostics (Krefeld, Germany). Anti-CD3 (Okt 3) was a gift from Thomas Noll (Forschungszentrum Jülich, Jülich, Germany). Dexamethasone was obtained from Sigma Chemie. Dexamethasone (crystalline) was dissolved in DMSO and aliquots of the stock solution (10−2 M) were stored at −20°C.

All other reagents were purchased from standard laboratory suppliers including Sigma-Chemie and Merck (Darmstadt, Germany), Rolipram, cilomilast, roflumilast (RPR-73401), RS-25344-000, and cilomilast were synthesized by the Chemical Department of Arzneimittelwerk Dresden GmbH (Radebeul, Germany) (now elbion AG). Blood from healthy volunteers was obtained in full compliance with ethical standards.

Determination of PDE4 Activity in Human Polymorpho-nuclear Leukocytes (PMNLs). PDE isoenzyme activity was determined in human PMNLs isolated from buffy coats. Peripheral blood from 12 healthy donors was collected and treated with 0.3% sodium citrate in PBS as anticoagulant. To remove the platelets, the cells were centrifuged at 700g for 20 min at room temperature. After aspiration of the platelet-rich supernatant, the residue was topped up to its initial volume with 0.13% sodium citrate in PBS. A volume of 45 ml was mixed with 5 ml of 5% dextran and allowed to stand for 1 h. After this dextran sedimentation, 20 ml of the supernatant was layered on the top of 15 ml of Ficoll-Paque and centrifuged for 30 min at room temperature. The sediments were resuspended and washed twice with 50 ml of PBS by centrifugation at 500g for 5 min at 4°C. The remaining red blood cells were lysed in 10 ml of a hypotonic buffer (155 mM NH4Cl, 10 mM NaHCO3, 0.1 mM EDTA, pH 7.4) for 6 min at 4°C. Afterwards, the cells were washed twice with 50 and 20 ml of PBS; this was followed by sonication of the cells. After centrifugation at 48,000g for 1 h the cytosolic PDE4 was present in the supernatant; this was stored at −20°C. PDE activity was determined in a one-step procedure in microtiter plates. The reaction mixture of 100 μl contained 50 mM Tris-Cl/5 mM MgCl2 buffer (pH 7.4), 100 μM cGMP, 0.5 μM [3H]cAMP, and the enzyme. Nonspecific enzyme activity was tested in the presence of 100 μM rolipram. The enzyme reaction was started by the addition of the substrate solution and was continued at 37°C for 30 min. It was stopped by the addition of 25 μl of YSI-SPA beads. One hour later, the radioactivity of the mixture was measured in a liquid scintillation counter (MicroBeta Trilux; PerkinElmer Life and Analytical Sciences, Boston, MA). A Biomek 2000 robot (Beckman Coulter Inc., Fullerton, CA) was routinely used for pipetting the incubation mixture. PDE4 activity was measured by quantification of [3H]5′-AMP, and concentrations that caused 50% inhibition (IC50) were determined using Hill equation.

IL-2, -4, -5, and TNFα Release from PBMCs. PBMCs (>90% lymphocytes) were extracted from heparinized blood of healthy donors by means of density centrifugation on Biocoll (density 1.077 g/ml). Cells were harvested and washed three times and then resuspended in RPMI 1640 medium supplemented with 10% FCS, HEPES 25 mM, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and 2-mercaptoethanol (50 μM). PBMCs were cultured in 96-well tissue-culture plates (1 × 104 cells/ml for IL-2, 2 × 104 cells/ml for IL-4/5, and 0.5 × 106 cells/ml for TNFα, 100 μl/well) at 37°C in a humidified atmosphere with 5% CO2 and 95% air for 18 h (IL-2 and TNFα) or 48 h (IL-4/5). Inhibitors were dissolved in DMSO, diluted in RPMI 1640 medium (supplemented), and added (in duplicate) to wells at a range of concentrations (final DMSO concentration, 0.1%; final concentrations of inhibitors, 0.01–10,000 nM). Viability was determined by trypan blue exclusion was uniformly >95%. Cells were stimulated as follows: 1) for IL-2 release, with 50 μl/well of stimulation agent, i.e., with phytohemagglutinin P (PHA-P, 20 μg/ml; Konno et al., 1994); 2) for IL-4/5 release, with concanavalin A (20 μg/ml; Endo et al., 1993), or anti-CD3 (1 μg/ml) plus anti-CD28 (0.5 μg/ml, applied in a solution); and 3) for release of TNFα, with LPS (1 μg/ml Salmonella abortus equi; Schindler et al., 1990). Each drug was preincubated for 30 min before stimulation. After incubation cells were pelleted, and IL-2, IL-4/5, or TNFα in the supernatants (fresh or frozen at −80°C) were measured by ELISA according to the manufacturer’s instructions.
Results

Effects of AWD 12-281 on IL-2, IL-4, IL-5, and TNFα Release of Stimulated Human PBMCs. AWD 12-281 was found to inhibit the PDE4 activity in human PMNLs with an IC50 of 9.71 ± 0.51 nM (mean ± S.E.M.). The compound inhibited the following processes in a concentration-dependent manner (Table 1): phytohemagglutinin-induced IL-2 release (EC50 of 50 nM), concanavalin A-induced IL-5 production (EC50 of 46 nM), anti-CD3 and anti-CD28 costimulated IL-4 release (EC50 of 121 nM), and IL-5 release (EC50 of 77 nM), and LPS-stimulated TNFα release in human PBMCs (EC50 of 88 nM). AWD 12-281 was compared with other PDE4 inhibitors (rolipram, RPR-73401, RS-25344-000, cilomilast, and rolflumilast). It could be shown that all these PDE4 inhibitors suppressed the release of IL-2, IL-4, IL-5, and TNFα in a concentration-dependent manner (Figs. 1–5). This activity could be correlated with the inhibitory activity of these compounds at the catalytic site of the PDE4 isozyme (Table 1, Fig. 6). The greatest inhibition, achieved at the highest concentration tested, amounted to about 90% for IL-2, IL-4, and IL-5 and about 70 to 75% for TNFα, with the exception of RS-25344-000 (82%), indicating that additional factors beside PDE4-dependent cAMP levels may contribute at least to the release of TNFα.

Despite the difference between the mechanisms of action of PDE4 inhibitors on the one hand and corticosteroid receptor activation on the other hand, dexamethasone also suppressed powerfully the release of IL-2, IL-4, IL-5, and TNFα. The inhibition by dexamethasone was likewise concentration-dependent and reached a similar maximum response (about 74% for TNFα and 90% for the interleukins). However, one difference is to be noted with regard to the release of IL-4. Although all PDE4 inhibitors tested up to a final concentration of 1 µM potently suppressed the IL-4 and IL-5 release when a costimulation protocol with anti-CD3 and anti-CD28 was followed (Figs. 3 and 4), these compounds showed no, or only a poor, inhibition of concanavalin A-stimulated IL-4 release. In contrast, dexamethasone prevented IL-4 release completely (Fig. 5).

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>PDE4 Inhibition IC50</th>
<th>IL-250 EC50</th>
<th>IL-550 EC50</th>
<th>IL-450 EC50</th>
<th>IL-450 EC50</th>
<th>TNFα50 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rolipram</td>
<td>75.50 (4.56) [4]</td>
<td>207.0 (43.82) [4]</td>
<td>338.6 (32.49) [2]</td>
<td>N.D.</td>
<td>N.D.</td>
<td>322.4 (87.50) [4]</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>17.80 (2.32) [4]</td>
<td>160.0 (33.03) [3]</td>
<td>165.3 (54.53) [2]</td>
<td>N.D.</td>
<td>N.D.</td>
<td>699.7 (179.64) [3]</td>
</tr>
<tr>
<td>RPR-73401</td>
<td>0.41 (0.075) [4]</td>
<td>2.6 (0.19) [3]</td>
<td>4.97 (0.78) [4]</td>
<td>N.D.</td>
<td>N.D.</td>
<td>5.4 (0.75) [4]</td>
</tr>
<tr>
<td>RS-25344-000</td>
<td>0.28</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.12 (1.94) [3]</td>
</tr>
<tr>
<td>Rolifumilast</td>
<td>0.27 (0.02) [8]</td>
<td>4.7 (0.88) [4]</td>
<td>3.4 (1.22) [4]</td>
<td>11.2 (0.78) [4]</td>
<td>21.2 (1.94) [3]</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>7.5 (0.51) [3]</td>
<td>2.9 (0.79) [4]</td>
<td>88 ± 1.6%</td>
<td>59 ± 4.2%</td>
<td>6.4 (1.42) [4]</td>
<td></td>
</tr>
</tbody>
</table>

Con A, concanavalin; N.D., not determined.

a PHA-P-stimulated.
b Concavalin A-stimulated.
c Costimulated.
d LPS-stimulated.
e Half-maximum inhibition.

Data from Alvarez et al. (1994).
ulated IL-4 release in human PBMCs (data not shown). As mentioned above, the concanavalin A-stimulated IL-5 release was powerfully suppressed (Fig. 2). Dexamethasone, on the other hand, was capable of suppressing not only the concanavalin A-induced release of IL-5 (Fig. 2) but also the release of IL-4 (regardless of the stimulation protocol used). Dexamethasone suppressed concanavalin A-stimulated IL-4 release with an IC$_{50}$ value of 9.9 ± 1.4 nM (mean ± S.E.M., four experiments, data not shown). The effect on the release of IL-4 induced by anti-CD3 and anti-CD28 was tested only at the concentration of 10 nM, which resulted in 59 ± 4.2% inhibition.

Inhibition of TNFα Release in Dispersed Human Polyp Cells. The in vitro effect of AWD 12-281 on TNFα release was investigated in allergically stimulated human dispersed nasal polyp cells; it was compared with the effects of the reference compounds rolipram, cilomilast, and roflumilast (Table 2). AWD 12-281 inhibited the allergically induced TNFα release with an EC$_{50}$ value of 111 nM. In comparison, rolipram, cilomilast, and roflumilast suppressed the
release of TNFα with EC₅₀ values of 156, 190, and 13 nM, respectively.

**Inhibition of TNFα Release in Human Whole Blood.**
The effect of AWD 12-281 on TNFα release was investigated and compared with that of rolipram, cilomilast, and roflumilast in human blood diluted 1:5 (Table 2). The selective PDE4 inhibitor AWD 12-281 suppressed the LPS-induced TNFα release in a concentration-dependent manner with an EC₅₀ of 900 nM. In comparison with effects of the reference compounds cilomilast (EC₅₀ of 1400 nM) and rolipram (EC₅₀ of 500 nM) AWD 12-281 was not a stronger inhibitor of LPS-induced TNFα release, despite its higher PDE4 inhibitory activity. The strongest inhibition in this assay was that by roflumilast, with an EC₅₀ of 17 nM.

**Discussion**
AWD 12-281 is a highly potent and selective PDE4 inhibitor that has been optimized for topical administration. The compound was shown to suppress the allergen-induced cell...
infiltration in bronchoalveolar lavage fluid of sensitized Brown Norway rats and the LPS-induced lung neutrophilia in Lewis rats, ferrets, and domestic pigs. In sensitized BP-2 mice, AWD 12-281 abolished the allergen-induced bronchial hyperresponsiveness (Kuss et al., 2003). In passively sensitized human airways, AWD 12-281 was shown to have a protective effect against allergen-induced contractions and to also have a weak relaxant effect on the spontaneous tone (Schmidt et al., 1999). These data indicate that AWD 12-281 is suitable for the treatment of asthma and COPD.

The aim of the current experiments was to evaluate the anti-inflammatory potential of AWD 12-281 in comparison with other PDE4 inhibitors and dexamethasone in various human inflammatory cell with the aim of predicting pharmacological activity in patients. The inhibitory power of AWD 12-281 on the PDE4 isoenzyme was found to be 9.71 nM

Fig. 6. Plots of linear regression (IL-2, dashed line; IL-5, solid line; TNFa, dotted line; p < 0.05) for inhibition of PDE4 versus inhibition of IL-2, IL-5, and TNFα release (EC_{50} values are given in Table 1).
TABLE 2
Inhibition of TNFα release in LPS-stimulated human blood, and inhibition of TNFα release in anti-IgE-stimulated human nasal polyp cells by AWD 12-281, rolipram, cilomilast, roflumilast, and dexamethasone

<table>
<thead>
<tr>
<th>Compound</th>
<th>TNFα, Whole Blood EC50a</th>
<th>TNFα, Polyp EC50b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>20 (4) [10]</td>
<td>1.3 (0.3) [4]</td>
</tr>
</tbody>
</table>

a EC50 estimated, limited number of data points.

b Half-maximum inhibition.

(\text{IC}_{50}) and was thus higher than that of cilomilast (17.8 nM) and lower than that of roflumilast (0.27 nM). Our data for the inhibition of PDE4 activity of the reference compounds are in agreement with literature data (Barnette et al., 1998; Giembycz, 2001; Hatzelmann and Schudt, 2001). On the basis of these data, AWD 12-281, like cilomilast and roflumilast, has the potential to be active in patients.

All PDE4 inhibitors investigated and dexamethasone were able to inhibit the release of IL-2, IL-4, IL-5, and TNFα from human PBMCs. Our data for rolipram, cilomilast, and rolipram are in agreement with published data from different sources using similar methods (Barnette et al., 1998; Hatzelmann and Schudt, 2001; Jimenez et al., 2001).

We could show that AWD12-281 and other PDE4 inhibitors suppressed anti-CD3/anti-CD28-stimulated release of IL-4, whereas they had little effect on concanavalin A-induced IL-4 release. Dexamethasone was capable of suppressing the IL-4 release induced by either of the two stimulation methods. In this context, T cell receptors (i.e., stimulation with anti-CD3/ anti-CD28) can be seen as a model for immunological diseases such as allergy and asthma. Stimulation with concanavalin A uses lectin receptors instead; this stimulation can be seen as nonspecific and not related to any particular disease. Although this difference does not help to identify the mechanistic basis for the difference in sensitivity to PDE4 inhibition, we can conclude that PDE4 inhibitors may be more selective than steroids and therefore especially suitable for the treatment of immunological diseases. However, the inhibition of IL-4 release by dexamethasone, but not by PDE4 inhibitors, may be also misleading, because a corresponding difference was not evident for the inhibition of IL-5 release.

If PDE4 inhibitory activity was plotted against inhibitory potential, a good correlation ($p < 0.05$) between PDE4 inhibitory activity of the inhibitors investigated and the inhibition of cytokine release in PHA-P-stimulated PBMCs (IL-2), concanavalin A-stimulated PBMCs (IL-5), and LPS-stimulated PBMCs (TNFα) could be shown (Fig. 6). This close correlation is suggestive. It indicates that the parameters measured are indeed closely related to PDE4-dependent cAMP levels, further supporting the belief that PDE4 inhibition is relevant for anti-inflammatory activity. The assays used here may therefore be used to predict the anti-inflammatory potential of new PDE4 inhibitors. Although the correlation between PDE4-inhibitory potential and inhibition of mediator release is very close for IL-2 and IL-5 release ($r^2 > 0.9$), it is somewhat lower for TNFα release ($r^2 = 0.82$). In addition, if we inspect the individual concentration-response curves (Fig. 5), we can see that the maximum TNFα inhibition achieved amounted to approximately 70 to 75% at 1 µM of each test compound. This was also shown by Hatzelmann and Schudt (2001), who found that the maximum inhibition of TNFα release in LPS-stimulated whole blood was only about 60 to 65%. This indicates that other factors besides PDE4 activity may contribute to TNFα release. Candidates are other PDE subtypes, i.e., PDE3 (Hatzelmann and Schudt, 2001) or PDE7 that are also cAMP-PDE isoenzymes and are found in activated T cells (Glavas et al., 2001; Smith et al., 2003). However, because the maximum suppression achieved with dexamethasone was also on the order of 70%, other factors besides PDE enzymes may also contribute to TNFα release.

We were interested in comparing the effects of AWD 12-281 on TNFα release in different cell preparations containing human inflammatory cells (i.e., PBMCs, whole blood, and nasal polyps) in relation to roflumilast and dexamethasone, to identify the preparation best suited to test PDE4 inhibitors. Cells dispersed from human nasal polyps differ from PBMCs and human whole blood in that inflammatory cells are derived directly from inflamed tissue. It can be expected that these cells are preactivated, owing to the ongoing inflammatory process. Furthermore, the stimulation mechanism used in the two preparations differs considerably. Although PBMCs and whole blood were stimulated according to standard methods, using bacterial endotoxin (LPS), which is a physiological stimulator only under conditions of bacterial infection and sepsis, we had established a stimulation protocol for nasal polyps that is closely related to an allergic reaction. The cells were first incubated with IgE; this was followed by the addition of anti-IgE according to the modified method described by the group of Bousquet (Campbell et al., 1993). Although the concentration for half-maximal inhibition for suppression of TNFα release was similar between PBMCs and dispersed nasal polyp cells for both PDE4 inhibitors tested, dexamethasone inhibited approximately 5 times more powerfully in human polyp cells (Tables 1 and 2). It should be noted that data reported previously in abstract form on TNFα release in whole blood and polyps (Heer et al., 1999, 2000; Kuesters et al., 1999) are based on the same row data but had been calculated using a (less suitable) in-house program.

The data indicate that PDE4 inhibitors can exert potent anti-inflammatory effects even in activated immune cells derived from inflamed cells by an allergy-related stimulation mechanism. The ability of the most potent PDE4 inhibitors to suppress TNFα were comparable with that of dexamethasone, which, on the basis of these data, was also active in the nanomolar range (1.3–20 nM) in suppressing TNFα release in PBMCs and nasal polyp cells. In contrast to these two cell preparations, the ability of AWD 12-281 to suppress LPS-induced TNFα release was approximately 1/10 as great in human whole blood. This difference may be related to the high plasma protein binding of AWD 12-281, which was found to be >99% (Kuss et al., 2003; D. Krone, personal communication, unpublished data). Isolated PBMCs as well as nasal polyp cells are cultivated in vitro in artificial media containing (after addition of both stimulation medium and compound solution) 10 or 9% FCS as the only source of plasma proteins. They therefore do not have the problem of binding the compounds to plasma proteins to the same extent as in whole blood. This may result in a reduced systemic
availability of the compound. However, because AWD 12-281 is optimized for topical administration, a high level of protein binding can contribute to the advantageous profile of AWD 12-281, i.e., long persistence in target tissue after administration by inhalation and low systemic availability (Kuss et al., 2003). This, in addition to the low oral bioavailability and rapid metabolism outside the target organ may result in a low emetic potential, especially after inhalative administration. Indeed, it could be shown that AWD 12-281 was exceptionally well tolerated and did not induce emesis, whereas being highly potent in animal models of inflammation when given via the inhalative route (Kuss et al., 2003). In addition, AWD 12-281 was found to have a low affinity to the rolipram binding site with an IC50 of 104 nM (Hoefgen et al., 1998). Because this site is discussed to be associated with emesis and gastrointestinal disturbances (Torphy, 1998), this may also contribute to the good tolerability found in vivo.

Comparing the three cell preparations to assess antiinflammatory potential, it can be concluded that PBMCs are well suited for the evaluation of the anti-inflammatory potential of PDE4 inhibitors. Although nasal polyp cells are more closely related to an ongoing inflammatory process, data obtained in this preparation did not differ from results obtained in PBMCs, at least with regard to PDE4 inhibitors. Whole blood in small amounts can be easily obtained from donors, without further preparation. This method is therefore also well suited for in vitro determination of TFNα release.

In summary, AWD 12-281 was shown to suppress powerfully the release of inflammatory mediators in three different human cell preparations. The ability of AWD 12-281 to inhibit PDE4 was found to be as great as that of other PDE4 inhibitors. These results, in combination with the potent activity of AWD 12-281 in animal models and the good separation between its emetic and anti-inflammatory activities, especially after topical administration, make AWD 12-281 an interesting candidate for the topical treatment of airway diseases such as asthma, COPD, and allergic rhinitis. Currently, phase II clinical studies are under way to evaluate the therapeutic potential of AWD 12-281 in these diseases.

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References


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