In Vivo Efficacy in Airway Disease Models of N-(3,5-Dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic Acid Amide (AWD 12-281), a Selective Phosphodiesterase 4 Inhibitor for Inhaled Administration

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ABSTRACT
N-(3,5-Dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide (AWD 12-281) is a highly potent and selective phosphodiesterase 4 (PDE4) inhibitor that was designed to have a metabolic profile that was optimized for topical administration. The aim of the current study was to explore the pharmacological profile of intratracheally administered AWD 12-281 in different models of asthma and chronic obstructive pulmonary disease (COPD) in comparison with steroids. To assess the anti-inflammatory potential of AWD 12-281, the antigen-induced cell infiltration in bronchoalveolar lavage fluid (BALF) of Brown Norway rats was determined. AWD 12-281 (ID50 of 7 μg/kg i.t.) as well as beclomethasone (0.1 μg/kg i.t.) suppresses late-phase eosinophilia when administered intrapulmonary. Furthermore, AWD 12-281 has also strong anti-inflammatory properties when tested in lipopolysaccharide-induced acute lung neutrophilia in Lewis rats (ID50 of 0.02 μg/kg i.t.), ferrets (ID50 of 10 μg/kg i.t.), and domestic pigs (2–4 mg/pig i.t. or 1 mg/kg i.v.). In pigs, AWD 12-281 was as effective as beclomethasone (0.4 mg/pig i.t.) and dexamethasone (0.28 mg/kg i.v.), although at 3 to 10 times the dosage. The bronchodilatory activity of AWD 12-281 was assessed in sensitized guinea pigs. AWD 12-281 (1.5 mg/kg i.t., 1-h pretreatment) inhibited allergen-induced bronchoconstriction by 68% (parameter airway resistance). In sensitized BP-2 mice AWD 12-281 abolished the allergen-induced bronchial hyperresponsiveness and eosinophilia in BALF, showing dose dependence. When given orally, i.v. or i.t., AWD 12-281 has a considerably lower emetic potential than cilomilast in ferrets and roflumilast in pigs. When given topically by inhalation, no emesis could be induced in dogs up to the highest feasible dose (15 mg/kg in 50% lactose blend). These results indicate that AWD 12-281 is a unique potential new drug for the topical treatment of asthma and COPD.

Emesis is the predominant side effect of PDE4 inhibitors, limiting their therapeutic potential. The signature side effects observed for PDE4 inhibitors are for the most part gastrointestinal, including nausea, vomiting, and increased gastric acid secretion. On the basis of results obtained in animals, it can be concluded that nausea and vomiting are produced through an action on the central nervous system, although there may also be a contribution from direct effects on the gastrointestinal system (Barnette, 1999). A number of highly potent PDE4 inhibitors are being developed for the treatment of asthma, COPD, rheumatoid arthritis, multiple sclerosis, and Crohn’s disease. Cilomilast (SB 207499; GlaxoSmithKline, Greenford, Middlesex, UK) and roflumilast (Altana Pharma Konstanz, Germany), the most advanced members of the class in phase III clinical trials, were reported to have a limited therapeutic window. Known PDE4 inhibitors

ABBREVIATIONS:
PDE4, phosphodiesterase type 4; COPD, chronic obstructive pulmonary disease; AWD 12-281, N-(3,5-dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide; BALF bronchoalveolar lavage fluid; LPS lipopolysaccharide; OVA ovalbumin; BAL bronchoalveolar lavage; BN Brown Norway; PBS phosphate-buffered saline; Cdyn lung dynamic compliance; Rl airway resistance; BHR bronchial hyperresponsiveness.
with emetogenic potential do not discriminate well between the catalytic (PDE4) and the rolipram binding site, respectively (Torphy, 1998), and experimental evidence suggests that this is related to the emetogenic potential of these compounds.

N-(3,5-Dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide (AWD 12-281; Fig. 1) is a potent and selective PDE4 inhibitor that was discovered and optimized in a program to identify potent inhibitors of PDE4 isoenzyme with increased tolerability over the existing compounds in clinical development. Early in vitro studies showed that AWD 12-281 suppresses the activity of many proinflammatory and immune cells that have been implicated in the pathogenesis of asthma and COPD (Marx et al., 2002). AWD 12-281 is currently in clinical development phase IIa for the treatment of asthma, COPD, and rhinitis.

The objective of the present study was to evaluate the therapeutic potential of AWD 12-281 in several preclinical models of airway diseases by intrapulmonary (intratracheal) drug administration. To assess the anti-inflammatory potential of AWD 12-281, the antigen-induced cell infiltration in bronchoalveolar lavage fluid (BALF) of Brown Norway rats was determined. The anti-inflammatory properties were also tested in lipopolysaccharide (LPS)-induced acute lung neutrophilia in Lewis rats, ferrets, and domestic pigs. The bronchodilatory activity and bronchial hyperreactivity of AWD 12-281 was assessed in sensitized guinea pigs and BP-2 mice. The emetic response of AWD 12-281 was investigated in sensitized guinea pigs and BP-2 mice. The tolerability in dogs was obtained from a 4-week inhalation toxicity study. Some of the data have been previously published in abstracts (Kuesters et al., 1999; 2000a; Poppe et al., 1999, 2000a–c; Poppe and Szelenyi, 1999; Marx et al., 1999; Kuss et al., 2002a–d).

Materials and Methods

All experiments were approved by the local authorities and were carried out according to the German animal protection law.

Animals. Male BP-2 mice (30–35 g b.wt., 8–16 weeks old) were obtained from Centre d’Elevage R. Janvier (Le Genest Saint-Isle, France). Male guinea pigs (210–560 g b.wt.) and male Lewis rats (280–300 g) were obtained from Charles River Wiga GmbH (Sulzfeld, Germany). Male Brown Norway rats (240–280 g) were obtained from Moellegaard Breeding and Research Centre A/S (Skensved, Denmark). Male and female ferrets (0.8–1.9 kg) were obtained from the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (Berlin, Germany). Male domestic pigs (10–15 kg b.wt., 8–10 weeks old) were purchased from a local breeder. Male and female purebred beagle dogs (6–6.5 months old) were obtained from Marshall Farms (North Rose, NY).

Chemicals. AWD 12-281, SB 207499, picamilast (RP 73401), and roflumilast were synthesized by Arzneimittelwerk Dresden GmbH (Radebeul, Germany). Beclomethasone dipropionate and LPS from Escherichia coli serotype 0111:B4 were purchased from Sigma Chemie (Deisenhofen, Germany). Bordetella pertussis vaccine concentrate (100 × 10⁶ heat-inactivated bacteria per milliliter) was purchased from Chiron Behring GmbH (Marburg, Germany). Aluminum hydroxide gel was obtained from Merck (Darmstadt, Germany). Lactose monohydrate (Pharmatose 325 M) lot 10015657 was purchased from DMV International (Veghel, The Netherlands). All other chemicals were of analytical grade.

Allergen-Induced Late-Phase Eosinophilia in the Bronchoalveolar Lumen of Actively Sensitized Brown Norway Rats. Male BN rats (BN/Mol, 200–250 g on delivery, 240–380 g at the end of the experiment) were actively sensitized by s.c. injections of a suspension of 10 µg of ovalbumin grade V (OVA) + 20 mg of Al(OH)₃ gel and i.p. B. pertussis vaccine (about 400 × 10⁹ heat-inactivated bacteria) on days 1, 14, and 21. On day 28, the eosinophilic inflammation was induced by inhaled allergen. The animals were exposed to a nebulized aerosol of OVA solution (10 mg of OVA/ml) in a nose-only inhalation system (TSE GmbH, Bad Homburg, Germany) for 60 min to provoke an influx of eosinophil granulocytes into the airways. Vehicle-treated control animals were exposed to a saline aerosol. The aerosol was generated by a nebulizer driven by compressed air at 0.2 MPa. Forty-eight hours later, i.e., at the time of maximal eosinophil influx, animals were sacrificed by urethane overdose (1.5 g/kg b.wt. i.p.), and BAL was performed with 3 × 4 ml of Hanks’ balanced solution at 37°C (average pooled recovery 9.0–10.5 ml of BALF per animal).

For detection of inhibitory potency, compounds were administered as single doses intratracheally 2 h before allergen challenge. In time-course studies, animals received test compounds or vehicle as single intratracheal doses at various administration times, either 1) prophylactically 24, 18, 6, or 2 h before allergen challenge; or 2) as therapeutic intervention 1, 2, 4, 6, 8, or 24 h after allergen challenge. For each time point, 6 to 19 drug-treated animals were used. Saline-challenged control rats and OVA-challenged vehicle rats were randomly distributed over all time points, but data from them were pooled for analysis.

For intratracheal drug administration, the animals were anesthetized (ketamine 20 mg/kg i.m. and xylazine 2.8 mg/kg i.m.), and a polyethylene catheter was inserted orally into the lower part of the trachea. The compounds were prepared as a dry powder formulation and blended with lactose to achieve 10 mg/kg b.wt. Powder. The dry powder formulation was filled into lockable one-way stopcocks placed between the tracheal catheter and a 5-ml syringe filled with air. The formulation was blown directly into the lung during the spontaneous inspiration phase in an air volume of 5 ml. The control animals received 10 mg/kg lactose.

Total cell count and the count of eosinophil granulocytes from BAL were determined with an automatic cell counter (Technicon H1E; Bayer Diagnostics GmbH, Munich, Germany). The cell count per animal was calculated from the number of cells per 1 µl of BAL multiplied by the BAL recovery in microliters per animal. For drug-treated and OVA-challenged animals, the percentage of inhibition of BAL eosinophilia and the reduction of total cell count in BAL fluid were calculated by the following formula:

\[
\% \text{ inhibition} = \frac{(\text{OVAC} - \text{SC}) - (\text{OVAD} - \text{SC})}{\text{OVAC} - \text{SC}} \times 100
\]
where SC is lactose-treated/saline-challenged control group; OVAC is lactose-treated/OVA-challenged control group, and OVAD is drug-treated/OVA-challenged group.

Data Analysis. Mean and S.E.M. were calculated for each parameter. For statistical evaluation, each group of actively treated animals was compared with the corresponding placebo-treated, saline-challenged, and OVA-challenged control groups by Kruskal-Wallis one-way analysis of variance on ranks. Multiple comparisons with the control group were performed by Dunn's method (Sigma Stat 2.0, version 1992–1997; SPSS Science, Chicago, IL). The percentage of inhibition for each dose was calculated. The ID₅₀ values were determined using an in-house programmed PC software package (EDX 2.1).

LPS-Induced Neutrophilia in the Bronchoalveolar Lumen of Lewis Rats. Conscious male Lewis rats (280–320 g) were exposed to an aerosol of lipopolysaccharide (100 μg/ml LPS and 0.1% hydroxyamine in phosphate-buffered saline; PBS) in a nose-only inhalation system (TSE GmbH) for 40 min to provoke an influx of neutrophil granulocytes into the airways. Vehicle-treated control animals were exposed to an aerosol of 0.1% hydroxyamine in PBS. Animals were sacrificed 6 h later by a urethane overdose, and a BAL was performed with 2 ml of Hanks’ balanced solution at 37°C (average pooled recovery 9.0–10.5 ml of BALF per animal).

Test compounds were administered intratracheally to Lewis rats under anesthesia, as described above, 2 h before the LPS provocation. Control animals received 10 mg/kg lactose i.t. Each group of animals treated with compounds was compared with control groups treated with vehicle, aerosolized with hydroxyamine/saline, or aerosolized with LPS in hydroxyamine. Each dose was tested in 3 to 14 animals. For determination of cell count in BALF as well as data analysis, see method described above (Allergen-Induced Late-Phase Eosinophilia in the Bronchoalveolar Lumen of Actively Sensitized Brown Norway Rats).

LPS-Induced Lung Neutrophilia in Ferrets. Male or female ferrets (0.8–1.9 kg) were anesthetized with 3% pentobarbital sodium (35–40 mg/kg i.p.) and intubated with a polyethylene tube. Individual ferrets were placed in a 5 liter Perspex chamber and exposed to a nebulized aerosol of LPS solution (100 μg/ml LPS and 0.1% hydroxyamine in phosphate-buffered saline; PBS) in a nose-only inhalation system (TSE GmbH) for 40 min to provoke an influx of neutrophil granulocytes into the airways. Vehicle-treated control animals were exposed to an aerosol of 0.1% hydroxyamine in PBS. Animals were sacrificed 6 h later by a urethane overdose, and a BAL was performed with 3 × 4 ml of Hanks’ balanced solution at 37°C (average pooled recovery 9.0–10.5 ml of BALF per animal).

Test compounds were administered intratracheally to Lewis rats under anesthesia, as described above, 2 h before the LPS provocation. Control animals received 10 mg/kg lactose i.t. Each group of animals treated with compounds was compared with control groups treated with vehicle, aerosolized with hydroxyamine/saline, or aerosolized with LPS in hydroxyamine. Each dose was tested in 3 to 14 animals. For determination of cell count in BALF as well as data analysis, see method described above (Allergen-Induced Late-Phase Eosinophilia in the Bronchoalveolar Lumen of Actively Sensitized Brown Norway Rats).

Data Analysis. The mean values and S.E.M. were used to express the data collected. The significance of differences was calculated by using Student’s t test to compare the neutrophil count from the BALF of animals given the same treatment and at the same point in the experiment with the corresponding normal control values (Sigma Stat 2.0, version 1992–1997; SPSS Science). The greatest amount of BALF practically possible was removed (recovery 6–17 ml). The BAL procedure took typically 0.5 to 1 min. Before being processed, the BALF recovered was kept at 4°C in 10-ml tubes containing 1.6 mg/ml potassium-EDTA (Sarstedt, Numbrecht, Germany) for a maximum of 2 h.

Directly after the first BAL, the substance was given, either i.v. or intrapulmonary. For intrapulmonary administration, the compounds were prepared as a dry powder formulation and blended with lactose to give a total of 30 mg of powder, with the exception of beclometasone (50 instead of 30 mg). The formulation was blown directly into the lung during the spontaneous inspiration phase in an air volume of 50 ml. The control animals received 30 mg of lactose per animal.

One hour or 0.5 h after treatment, the pig was exposed to 20 min of ultrasound-nebulized LPS aerosol generated from a 300-ml solution of 16.7 μg of LPS per 1 ml of 0.1% hydroxyamine solution (nebulizer, Heyer USE77; Carl Heyer, Bad EMG, Germany). Four and 6 h after LPS provocation, further BALF samples were taken and treated as described above.

Respiratory depression through anesthetic administration had to be avoided by careful application of the anesthetic. The effect of each compound on the BALF neutrophil count (cells per BAL recovery) was calculated from the average neutrophil count of the LPS-challenged control animal BAL, the compound BAL, and the normal BAL before LPS challenge in the following way:

% inhibition neutrophilia = (LPS control – compound) × 100

Data Analysis. The mean values and S.E.M. were used to express the data collected. The significance of differences was calculated by using Student’s t test to compare the neutrophil count from the BALF of animals given the same treatment and at the same point in the experiment with the corresponding normal control values (Sigma Stat 2.0, version 1992–1997; SPSS Science).

Allergen-Induced Bronchoconstriction in the Anesthetized, Mechanically Ventilated Guinea Pig. Male guinea pigs (240–560 g b.wt.) were actively sensitized with OVA [20 μg and 20 mg Al(OH)₃ dissolved in 0.5 ml of saline] intraperitoneally on two consecutive days. Two weeks later, these animals were used for experiments.

They were anesthetized with 1,500 mg/kg i.p. urethane. A catheter was placed in the right jugular vein. The left carotid artery was prepared, and a catheter for continuous blood pressure and heart rate measurement was inserted. The trachea was cannulated and connected to a small-animal respirator (KTR 5; FMI, Engelsbach, Germany). The animals were artificially respirated with a mixture of 10% oxygen and ambient air (60/min, 15 ml/kg b.wt.). To exclude spontaneous breaths, animals were paralyzed with 10 mg/kg i.v. gallamine.

The respiratory flow was measured with a Fleisch tube (size 0000) in line with the respirator. The transpulmonary pressure was recorded continuously through a side connection in the tubes (inflation pressure) and an intrathoracic cannula (intrathoracic pressure). From these data, lung dynamic compliance (Cdyn) and airway resistance (Rl) were calculated for each respiratory cycle and stored in a computer-controlled system (PMS PR 800; Munmed Systems Ltd., London, UK).

After preparation and an equilibration period of 10 min, base level 1 was noted. The animals then received AWD 12-281 (1.5 mg/kg i.t.) as dry powder formulation blended with lactose to achieve an administration volume of 10 mg/kg. For drug administration, the blend was filled into lockable one-way stopcocks. The artificial respiration was shortly interrupted and the stopcock was placed between the tracheal catheter and a 10-ml syringe filled with air. The formulation was blown directly into the lung in an air volume of 5 to 7 ml, dependent on the body weight of the

In Vivo Efficacy of AWD12-281, a Selective PDE4 Inhibitor 375
animals. The control animals received 10 mg/kg lactose. After drug administration, the animal was reconnected with the respirator, and the respiratory and cardiovascular parameters were continuously recorded. Thirty minutes or 1 h after drug administration, all animals underwent broncho-alveolar lavage. The extent of exposure was checked by measuring both the mean respiratory minute volume and the content of AWD 12-281 aerosol in

**Data Analysis.** Mean and S.D. were calculated for each parameter and treatment group. The significance of differences between sensitized and OVA-challenged control animals was determined by one-way analysis of variance followed by Dunnett’s test (Sigma Plot 4.0; SPSS Science). A value of $p < 0.05$ was considered to be statistically significant. Significant changes ($p < 0.05$) between sensitized and OVA-challenged control groups are marked with an asterisk.

**Emetic Effects in Conscious Domestic Pigs.** Young, healthy, male domestic pigs (6–10 kg) were purchased from a local breeder. They were housed under constant environmental conditions. When the experiment started, the pigs weighed 7 to 30 kg. For drug administration, the pigs were slightly anesthetized with intramuscular ketamine/xylazine (10 mg/kg ketamine and 2 mg/kg xylazine). Drug administration was performed either intravenously through an ear vein or orally through an esophageal tube directly into the stomach. After drug administration, the animals were placed in individual observation cages of about 4 m². On average, the animals recovered from the anesthesia within 20 min. The pigs were then observed for 8 h. Emesis was characterized by the vomiting of solid or liquid material. Every hour, an observer recorded the number of vomits (direct observation or by counting the number of vomits on the floor of the cage) and the behavioral changes hourly.

Data are expressed as the fraction of animals that responded with emesis of the total number tested per dose. The first emetic dose was defined as the dose at which the first vomiting with expulsion of solid or liquid material could be induced in at least one of three animals tested.

**Emetic Potential of AWD 12-281 in Dogs with 4-Week Inhalative Administration.** After at least 2 weeks of acclimatization, purebred beagle dogs (6–6.5 months old; Marshall Farms) were randomized to four groups (three males and three females for low- and mid-dose group, five males and five females for vehicle and high-dose group). The animals were exposed once daily to AWD 12-281 in a 50% lactose blend by inhalation (smoke only exposure with masks, 45-min exposure time) at target doses of 0.6, 3, and 15 mg/kg; the vehicle group was exposed to lactose under similar conditions. The aerosol was generated using a piston/brush feed aerosol generator (Broillet; CR Équipements S.A., Tannay/Switzerland or Palas GmbH, Karlsruhe/Germany for lactose control) and discharged constantly into the individual masks of the dogs. A micronizing jet mill was included into the conduction system between the aerosol generator and the exposure chamber.

The extent of exposure was checked by measuring both the mean respiratory minute volume and the content of AWD 12-281 aerosol in the trachea into the lung and immediately recovered. This procedure was repeated five times (average pooled recovery from each animal 3.2 ml of BALF). For determination of cells in BALF, see “Allergen-Induced Late-Phase Eosinophilia in the Bronchoalveolar Lumen of Actively Sensitized Brown Norway Rats” (above).
the respired air. The duration of exposure was adjusted individually to compensate latest group mean body weight during the experiment. To ensure lung exposure, the particle size of the aerosol was measured. The mass median aerodynamic diameter and geometric standard deviation determined gravimetrically was 3.49 μm (geometric standard deviation of 2.47) for the high-dose group. The particle size was similar or even smaller for the other dose groups and is within the respirable range. Dogs were fed after completion of exposure of all animals. The aim of this study was to assess the toxicological profile of AWD 12-281 after high-dose inhalative administration. The dogs were closely observed during the exposure time as well as for at least 2 h after the end of the exposure for the occurrence of emesis as well as for signs of nausea and other behavioral abnormalities. On the 1st day and on day 27, plasma was withdrawn before start of the inhalation as well as at 10 min, and 1, 3, 6, and 24 h after the end of the inhalation period to determine the extent of exposure.

**Results**

**Inhibition of Allergen-Induced Late-Phase Eosinophilia**

Inhalative OVA challenge in actively OVA-sensitized BN rats leads to a significant increase in both the total cell count and eosinophil count in the BALF 48 h after allergen challenge, in comparison with the BALF obtained from OVA-sensitized saline-challenged BN rats. This indicates an allergen-induced eosinophilic inflammation.

The effect of the selective PDE4 inhibitor AWD 12-281 on the allergen-induced late-phase eosinophilia was investigated in comparison with beclomethasone, a glucocorticoid. Test compounds (dry powder formulations) were administered intrapulmonarily 2 h before allergen challenge. Treatment of animals with single doses of AWD 12-281 in the range 1 to 30 μg/kg i.t. caused a significant and dose-depen-

![Fig. 2. A and B, allergen-induced eosinophilia in BN rats was used as a symptom model of eosinophilic lung inflammation in bronchial asthma. A, effect of single doses of AWD 12-281 (1–30 μg/kg i.t.) (gray columns; n = 5–15 animals/group) administered 2 h before allergen challenge on OVA-induced eosinophilia in BN rat’s BAL, taken 48 h after ovalbumin aerosol exposure, respectively. The ID50 value of AWD 12-281 was 7 μg/kg i.t. B, effect of single doses of beclomethasone dipropionate (0.01–1 μg/kg i.t.) (hatched columns; n = 11–13 animals/group) administered 2 h before allergen challenge on OVA-induced eosinophilia in BN rat’s BAL, taken 48 h after ovalbumin aerosol exposure, respectively. Beclomethasone at the doses of 0.01 to 1 μg/kg i.t. inhibited the eosinophilia by approximately 50%. SC, lactose-treated/saline-challenged normal control group (white column; n = 14–15); OAC, lactose-treated/OVA-challenged control group (black column; n = 28–31). Data are shown as mean ± S.E.M. **, p < 0.01 in a comparison with normal control; *, p < 0.05 and **, p < 0.01 in a comparison with lactose-treated. OVA-aerosol-challenged animals (OVA control). The result is summarized from four separate tests each.
dent inhibition of BAL eosinophilia (ID$_{50}$ of 7 µg/kg i.t.). Beclomethasone at the doses of 0.01 to 1 µg/kg i.t. inhibited the eosinophilia by approximately 50% (Fig. 2, A and B).

**AWD 12-281 Time-Course Study**

The aim of this study was to investigate the duration of anti-inflammatory action of AWD 12-281 in an allergic asthma model using actively sensitized BN rats. In addition, we tested whether AWD 12-281 was able to suppress eosinophilic inflammation if administered after the allergen challenge (therapeutic intervention). The compound was given to rats i.t. at various times before and after allergen challenge. AWD 12-281 30 µg/kg was compared with the steroid beclomethasone (1 µg/kg, also i.t.). Eosinophilia and total cell count in the bronchoalveolar space were investigated by using BAL at the time of maximal influx of eosinophils (48 h after allergen challenge).

The treatment of animals with single doses of 30 µg/kg i.t. AWD 12-281 caused a significant reduction of antigen-induced increase in eosinophil count in BALF at different times ranging from −18 h to +8 h, relative to allergen challenge, by 54 and 35%. When AWD 12-281 was given at the times −24 h and +24 h, relative to allergen challenge, a slight, but not statistically significant reduction in eosinophil count (by 26–31%) was measured. Within the prophylactic treatment groups (−18 to −2 h) the allergen-induced increase in the total cell count in BAL fluid showed a reduction by 30 to 50% (not statistically significant by Dunn’s method).

Beclomethasone dipropionate, at a dose of 1 µg/kg i.t., was used as reference. The treatment of animals with of 1 µg/kg i.t. beclomethasone caused a significant inhibition of the antigen-induced increase in eosinophil count in BALF at the time points 6 and 2 h before allergen challenge; inhibition was by 46 and 51% ($p < 0.05$), respectively. In the “−6-h” versus saline-challenged plus lactose, $p < 0.05$ and $p < 0.01$. * * *, statistical significance in a comparison with OVA-challenged plus lactose, $p < 0.05$ and $p < 0.01$. Data are presented as mean ± S.E.M. The result is summarized from five separate tests.
treatment group, the allergen-induced increase in the total cell count in BALF was abolished resulting in a 100% reduction ($p < 0.05$; data not shown).

**Inhibition of LPS-Induced Neutrophilia**

Inhalation of lipopolysaccharide in Lewis rats resulted in an acute inflammatory reaction characterized by a strong increase in the number of neutrophils and in the total cell count (data not shown). The maximum neutrophil cell count was previously found 6 h after LPS provocation. In this model, compounds were tested by intratracheal drug dry powder administration 2 h before exposure to LPS. The intrapulmonary treatment of animals with single doses of AWD 12-281 (micronized compound blended with lactose carrier) in the range 0.001 to 100 μg/kg (Fig. 4, A and B) caused a significant and dose-dependent inhibition of BAL neutrophilia ($ID_{50}$ of 0.02 μg/kg i.t.). Intrapulmonary beclomethasone in the range 0.001 to 0.1 μg/kg showed a strong anti-inflammatory effect ($ID_{50}$ of 0.007 μg/kg i.t.; Fig. 5).

In LPS-aerosol-challenged ferrets, as in rats, significant increases in neutrophil and total cell counts were detected in the BALF 6 h after LPS provocation (data not shown). Treatment of animals 2 h before challenge with a single dose of 1, 3, or 10 mg/kg i.p. AWD 12-281 caused a dose-dependent inhibition of BAL neutrophilia (18, 64, and 78%, respectively) with an estimated $ID_{50}$ value of 2.4 mg/kg i.p. (data not shown). RP 73401 also strongly inhibited pulmonary neutrophil accumulation in ferrets. Treatment of animals 2 h before and 1 h after LPS exposure with 2 × 0.5 mg/kg i.p. RP 73401 led to virtually complete (99%) suppression of neutrophilia. A single oral dose of 30 mg/kg AWD 12-281 in one animal caused an inhibition of LPS-induced neutrophilia by 95%, indicating oral activity. AWD 12-281 was as effective as the reference compound SB 207499 (10 mg/kg p.o.; 73%) and RP 73401 (10 mg/kg p.o.; 25%; Dale et al., 1995) after oral administration. When AWD 12-281 (in the range 1–100 μg/kg) was administered intrapulmonarily as a dry powder 2 h before LPS exposure, a strong and dose-dependent anti-inflammatory effect against lung neutrophilia was detected ($ID_{50}$ of approx. 0.01 mg/kg i.t.; Table 1).

As a third animal model of neutrophilic inflammation, young domestic pigs were used. In these animals, significant increases in neutrophil and total cell counts in the BALF were induced by inhalative LPS-aerosol challenge (data not shown) and could be detected 4 and 6 h after administration of LPS. Intravenous application of 1 mg/kg AWD 12-281 30 min before the start of LPS challenge resulted in a reduction of neutrophilia by 70% at both 4 and 6 h ($p < 0.001$; Fig. 6A; Table 1). Dexamethasone (0.28 mg/kg i.v.) significantly inhibited the BAL neutrophilia at 4 and 6 h (by 80 and 90%; Fig. 6B).

AWD 12-281 was also tested after topical administration in pigs. The lowest dose (0.4 mg/animal i.t.) inhibited the LPS-induced neutrophilia in domestic pigs by 50% at 4 h with a statistical significance of $p < 0.01$. The same treatment had no effect at 6 h. At 1 mg/animal i.t., the 4-h value was 63% inhibition ($p < 0.01$), whereas at 6 h 21% inhibition was achieved (no statistical significance). This anti-inflammatory
Inhibition of Allergen-Induced Bronchoconstriction

The inhaled allergen (OVA) challenge as aerosol resulted in a marked bronchoconstriction in placebo-treated, actively sensitized guinea pigs (Table 2). The $C_{dyn}$ decreased immediately by 75 to 80% (control groups 1 and 2). The marked fall of compliance leads to a strong increase in $R_l$. The rises in $R_l$ were calculated to be 124 and 162% (control groups 1 and 2, respectively).

The differences in the degree of early phase bronchospasm after allergen challenge in the animals of both control groups lie within the variability of the method. Furthermore, the results were independent of the time of intratracheal vehicle (lactose) administration (30 min or 1 h before OVA challenge). AWD 12-281 at a dose of 1.5 mg/kg had strong and significant bronchodilatory effects when administered intratracheally as a dry powder formulation with lactose carrier 1 h before OVA challenge. The OVA-induced decrease in $C_{dyn}$ was inhibited by 24% compared with the control group ($p < 0.01$) and the $R_l$ was strongly inhibited, by 68% compared with the control group ($p < 0.01$). With a shorter pretreatment time (30 min), the bronchodilatory effect could not be shown. No inhibitory effect was observed after systemic administration using the oral and intraperitoneal routes and doses up to 30 mg/kg AWD 12-281 (2 h before allergen challenge; data not shown).

Inhibition of BHR and Lung Eosinophilia

The effect of AWD 12-281 was investigated on allergen-induced BHR in response to methacholine and airway inflammation in actively sensitized BP-2 mice. Allergen-challenged, sensitized control mice react with an increase in airway resistance at lower doses of methacholine in comparison with naive control mice. The repeated administration of AWD 12-281 (five administrations) during intranasal OVA challenge, in the dose range 10 to 60 mg/kg i.p., reduced BHR against methacholine as well as the increase in total cell count and eosinophil number in BALF in a dose-dependent manner. The effect of 30 mg/kg SB 207499 i.p. (five administrations) was directly comparable with that observed in the AWD 12-281 treatment group with the same dose. Dexamethasone (5 mg/kg i.p., five administrations) had a weak effect on BHR, but completely suppressed the influx of eosinophils into the airways (Tables 3 and 4).

Induction of Emesis in Ferrets

Emesis is a dose-limiting side effect of currently available PDE4 inhibitors. Owing to the animal's physiology, emesis cannot be induced in rodents, but it can be in ferrets, dogs, and domestic pigs. We have selected the ferret as an accepted animal model to evaluate the potential to induce emesis of AWD 12-281. Overall, the intraperitoneal administration of AWD 12-281 was well tolerated in doses below 15 mg/kg. At a dose of 15 mg/kg AWD 12-281, in one of four ferrets a single vomit was observed 9 min after drug administration. This dose was defined as the minimal emetic dose for the i.p. route. AWD 12-281 (20 mg/kg) induced in three of nine ferrets significant emesis (average 3.0 vomits per animal, mostly within 1 h). In one of three animals, retching was observed as well as vomiting. SB 207499, at 1 mg/kg i.p., was well tolerated in all animals. At 3 mg/kg, emesis was observed in one of four ferrets 5 to 40 min after drug administration (one occurrence of retching after 5 min and six vomits within 40 min).

In a second series of experiments, the oral route was tested. The oral doses of 10 and 20 mg/kg AWD 12-281 were well tolerated without any signs of emesis. At a dose of 30 mg/kg AWD 12-281, two occurrences of mouth scratching and retching were observed in one of three ferrets, each beginning 45 min after drug administration and lasting approximately 2 min (minimal emetic dose). In one of three ferrets given AWD 12-281 at 40 mg/kg, emesis but no retching occurred, starting 112 min after drug administration (Table 1). Another of the three ferrets exhibited a long period (130 min) of intermittent retching and scratching, starting 55 min after

---

**Table 1**

<table>
<thead>
<tr>
<th>Ferrets</th>
<th>AWD 12-281</th>
<th>SB 207499</th>
<th>RP 73401</th>
</tr>
</thead>
<tbody>
<tr>
<td>First emetic dose p.o.</td>
<td>40 mg/kg</td>
<td>10 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Neutrophilia ID$_{50}$ p.o.</td>
<td>~10 mg/kg</td>
<td>~10 mg/kg</td>
<td>10 mg/kg; 25% inhibition$^a$</td>
</tr>
<tr>
<td>Neutrophilia ID$_{50}$ i.t.</td>
<td>0.01 mg/kg</td>
<td>N.I.</td>
<td>N.I.</td>
</tr>
<tr>
<td>Ratio p.o.</td>
<td>~4</td>
<td>~1</td>
<td>~1</td>
</tr>
<tr>
<td>Ratio i.t.</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Domestic Pigs</th>
<th>AWD 12-281</th>
<th>Rolipram</th>
<th>Roflumilast</th>
</tr>
</thead>
<tbody>
<tr>
<td>First emetic dose i.v.</td>
<td>9 mg/kg</td>
<td>0.3 mg/kg</td>
<td>0.3 mg/kg</td>
</tr>
<tr>
<td>Neutrophilia effective dose i.v.</td>
<td>&lt;1 mg/kg</td>
<td>N.I.</td>
<td>N.I.</td>
</tr>
<tr>
<td>Neutrophilia effective dose i.t.</td>
<td>~0.1–0.25 mg/kg</td>
<td>&gt;9</td>
<td>&gt;9</td>
</tr>
<tr>
<td>Ratio i.v.</td>
<td>&gt;9</td>
<td>&gt;9</td>
<td>&gt;9</td>
</tr>
<tr>
<td>Ratio i.t.</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

N.I., not investigated.
$^a$ Data from Dale et al., 1995.
drug administration. The observed average number of vomits was 1.0. SB 207499, at a dose of 3 mg/kg i.v., did not induce emesis in any of the three ferrets studied. At a dose of 10 mg/kg, two of six ferrets exhibited emesis, the two ferrets had an average of 2.5 vomits each. RP 73401 at the doses of 1 and 3 mg/kg p.o. was free of any emetic side effects. At an oral dose of 10 mg/kg, two of two ferrets exhibited severe emesis, combined with retching and backward walking.

Induction of Emesis in Domestic Pigs

As a second model, the young domestic pig was used. Overall, the i.v. administration of AWD 12-281 in young domestic pigs was well tolerated up to 12 mg/kg, but at 9 and 12 mg/kg i.v. in one of four pigs a single vomit was observed 5 and 2 h after drug administration, respectively. In the other pigs, slight salivation and chewing were observed. The minimal emetic dose was set at 9 mg/kg i.v. in five pigs, the intrapulmonary administration of 100 mg of AWD 12-281 was not emetic (Table 1). Emetic doses of rolipram were 0.3 mg/kg i.v. and 3 mg/kg p.o. in pigs. SB 207499 (cilomilast), when given at doses of 3 and 6 mg/kg i.v., was not emetic. At the dose of 9 mg/kg, emesis was observed in one of four pigs (twice within 2 h). SB 207499 at 12 mg/kg i.v. induced significant emesis in three of six pigs (average 2.7 vomits per animal with observed emesis, mostly within 1 h). Roflumilast, when given at a dose of 0.1 mg/kg i.v. to four animals, was not emetic, but all animals made chewing movements with their mouths, including grinding their teeth, 3 h after drug administration. At the dose of 0.3 mg/kg i.v., a single occurrence of emesis was observed in one of five pigs. During the intravenous injection of the dose, 1.0 mg/kg, all animals showed a short increase in respiratory rate and a general restlessness. At this dose, roflumilast (1.0 mg/kg i.v.) induced significant emesis in all of the four animals tested, starting 10 to 15 min after dose administration (average 3.75 vomits per animal, mostly within 1 h).

Emetic Potential of AWD 12-281 in Dogs

In the 4-week inhalation study in the dog the mean achieved doses were 0.58, 3.00, and 14.05 mg/kg/day for the three dose groups. The dose of 14.05 mg/kg was technically the highest achievable dose with micronized compound in a 50% lactose blend. In this study, AWD 12-281 was very well tolerated. No emesis and no signs of nausea were observed neither during the exposure nor during the observation period after the exposure in all dose levels throughout the study. On day 27, the high dose was associated with mean plasma AWD 12-281 $C_{\text{max}}$ values of 428 ng/ml in males and 696 ng/ml in females.

Discussion

The objective of this study was to establish a comprehensive profile in vivo of the airway pharmacology of AWD 12-
TABLE 2
Inhibitory effects of intrapulmonary (i.t.) AWD 12-281 on allergen-induced late phase bronchoconstriction in the guinea pig

In vivo effects of i.t. administered AWD 12-281 (dose 1.5 mg/kg, 0.5 or 1 h before antigen challenge) on the OVA-induced fall of lung dynamic compliance ($C_{dyn}$) and the according rise of airway resistance ($R_L$) in actively sensitized guinea pigs. Controls received the vehicle (lactose, Pharmatose 325 M, 10 mg/kg I.t.). Results represent percentage of inhibition with respect to controls. Data are given as mean ± S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$C_{dyn}$</th>
<th>$R_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall</td>
<td>Inhibition of the OVA induced fall</td>
</tr>
<tr>
<td>Control group 1</td>
<td>75 ± 11</td>
<td>0</td>
</tr>
<tr>
<td>Pharmatose 325 M, 10 mg/kg b.w. I.t., 1 h before OVA</td>
<td>79 ± 6 N.S.</td>
<td>-5</td>
</tr>
<tr>
<td>AWD 12-281, dose 1.5 mg/kg I.t., as dry powder formulation, 1 h before OVA</td>
<td>80 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>Control group 2</td>
<td>61 ± 14⁺</td>
<td>24</td>
</tr>
</tbody>
</table>

* Statistical significance versus OVA challenged control group $p < 0.01$.

TABLE 3
Inhibitory effects of repeated i.p. AWD 12-281 on BHR in the BP-2 mouse

In vivo effects of intraperitoneal AWD 12-281 in comparison with SB 207499 and dexamethasone on methacholine-induced rise of airway resistance (PD90 and PD150) in actively sensitized BP-2 mice. Sensitized animals react at smaller doses of methacholine i.v., indicating airway hyperresponsiveness. Controls received the vehicle (0.5% dimethyl sulfoxide in saline i.p.; 0.2 ml/30 g b.w.). Results represent percentage of inhibition of induced increase in total cell count and eosinophils of sensitized BP-2 mice. Sensitized and allergen-challenged animals showed high counts of total cells and eosinophils in BALF, indicating late phase eosinophilia. Controls received the vehicle (0.5% dimethyl sulfoxide in saline i.p.; 0.2 ml/30 g b.w.). Results represent percentage of inhibition of induced increase in total cell count and eosinophils vs. sensitized animal controls. Data are given as mean ± S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PD90</th>
<th>Increase of MCh, Dose</th>
<th>PD150</th>
<th>Increase of MCh, Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/kg MCh i.v.</td>
<td>%</td>
<td>µg/kg MCh i.v.</td>
<td>%</td>
</tr>
<tr>
<td>Controls</td>
<td>56.0 ± 4.2*</td>
<td>70</td>
<td>21.1 ± 6.9</td>
<td>70</td>
</tr>
<tr>
<td>Naive animals</td>
<td>15.1 ± 4.2*</td>
<td>70</td>
<td>27.2 ± 8.7 N.S.</td>
<td>70</td>
</tr>
<tr>
<td>Sensitized animals</td>
<td>10.4 ± 4.2</td>
<td>0</td>
<td>22.1 ± 6.9</td>
<td>0</td>
</tr>
<tr>
<td>AWD 12-281</td>
<td>12.0 ± 5.0 N.S.</td>
<td>15</td>
<td>21.1 ± 7.1 N.S.</td>
<td>0</td>
</tr>
<tr>
<td>5 × 10 mg/kg i.p.</td>
<td>14.6 ± 5.9 N.S.</td>
<td>40</td>
<td>24.1 ± 7.3 N.S.</td>
<td>9</td>
</tr>
<tr>
<td>5 × 30 mg/kg i.p.</td>
<td>25.9 ± 15.5⁺</td>
<td>149</td>
<td>41.9 ± 16.5**</td>
<td>190</td>
</tr>
<tr>
<td>SB 207499 (Cilomilast)</td>
<td>13.9 ± 3.8 N.S.</td>
<td>34</td>
<td>23.3 ± 6.6 N.S.</td>
<td>5</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>13.6 ± 3.7 N.S.</td>
<td>31</td>
<td>21.1 ± 6.8 N.S.</td>
<td>0</td>
</tr>
<tr>
<td>5 × 5 mg/kg i.p.</td>
<td>13.6 ± 3.7 N.S.</td>
<td>31</td>
<td>21.1 ± 6.8 N.S.</td>
<td>0</td>
</tr>
</tbody>
</table>

MCh, methacholine; * and **, statistical significance versus sensitized animals (unpaired Student's t test) $p < 0.05$; $p < 0.01$.

TABLE 4
Inhibitory effect of repeated i.p. AWD 12-281 on allergen-induced increase in airway total cell count and eosinophilia in BP-2 mice

In vivo effects of i.p. AWD 12-281 in comparison with SB 207499 and dexamethasone on OVA-induced increase in total cell count and eosinophilia in BALF in actively sensitized BP-2 mice. Sensitized and allergen-challenged animals showed high counts of total cells and eosinophils in BALF, indicating late phase eosinophilia. Controls received the vehicle (0.5% dimethyl sulfoxide in saline i.p.; 0.2 ml/30 g b.w.). Results represent percentage of inhibition of induced increase in total cell count and eosinophils vs. sensitized animal controls. Data are given as mean ± S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Cell Count</th>
<th>Inhibition of Induced Increase in TCC</th>
<th>Eosinophils</th>
<th>Inhibition of Induced Increase in EOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mill./animal</td>
<td>%</td>
<td>Mill./animal</td>
<td>%</td>
</tr>
<tr>
<td>Controls:</td>
<td>8</td>
<td>0.768 ± 0.137</td>
<td>-</td>
<td>0.046 ± 0.034</td>
</tr>
<tr>
<td>Naive animals</td>
<td>8</td>
<td>2.223 ± 0.747*</td>
<td>0</td>
<td>1.118 ± 0.633*</td>
</tr>
<tr>
<td>Sensitized animals</td>
<td>8</td>
<td>1.895 ± 0.503 N.S.</td>
<td>15</td>
<td>0.955 ± 0.502 N.S.</td>
</tr>
<tr>
<td>AWD 12-281</td>
<td>10 mg/kg i.p.</td>
<td>8</td>
<td>1.278 ± 0.310**</td>
<td>57</td>
</tr>
<tr>
<td>30 mg/kg i.p.</td>
<td>7</td>
<td>1.376 ± 0.250*</td>
<td>38</td>
<td>0.040 ± 0.017***</td>
</tr>
<tr>
<td>SB 207499 (Cilomilast)</td>
<td>30 mg/kg i.p.</td>
<td>8</td>
<td>1.342 ± 0.387 N.S.</td>
<td>40</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>5 mg/kg i.p.</td>
<td>8</td>
<td>1.484 ± 0.464 N.S.</td>
<td>33</td>
</tr>
</tbody>
</table>

TCC, total cell count; EOS, eosinophils; *, statistical significance versus naive animals $p < 0.05$; **, and ***, statistical significance versus sensitized animals (unpaired Student's t test) $p < 0.05$; $p < 0.01$; $p < 0.001$.

281, a novel PDE4 inhibitor that has been optimized for topical (inhalative) administration. Therefore, we evaluated its anti-inflammatory, antiallergic, and bronchodilatory potential in several disease models in guinea pigs, mice, rats, ferrets, and domestic pigs, in direct comparison with the glucocorticoids dexamethasone and beclomethasone. Addi-
tionally, we chose other PDE4-specific compounds as reference drugs, such as piclamilast (RP 73401; Ashton et al., 1994; Raeburn et al., 1994) and roflumilast (Bundschuh et al., 2001). To compare the safety margin of AWD 12-281 in ferrets and domestic pigs, we used rolipram and RP 73401 as the archetypal PDE4 inhibitors (Underwood et al., 1993), and cilomilast and roflumilast as the PDE4 inhibitors most advanced in clinical development (Barnette, 1999; Burnouf and Pruniaux, 2002).

Inhibitors of the isoenzyme PDE4 are effective inhibitors of the recruitment of eosinophils into sites of allergen-induced inflammation in BN rat lung (Howell et al., 1995). These studies suggest that the BN rat model of late-phase eosinophilia is a useful tool in the screening and development of selective PDE4 inhibitors for the treatment of allergic lung inflammation. Although eosinophilic inflammation is a principal symptom of allergic inflammation, the total cell count is another indicator of the induced inflammatory process (Djukanovic et al., 1990; Tarayre et al., 1992). AWD 12-281 suppressed potently the eosinophilic inflammation if administered topically at low doses. The extent of this effect was similar to that of beclomethasone, however, at higher doses. Because AWD 12-281 is being developed for inhalative administration, we were interested in evaluating the duration of action after i.t. administration. We selected the eosinophilic inflammation in OVA-sensitized BN rats as a model, and an intrapulmonary dose level of 30 μg/kg, because this dose was shown to reduce inflammation reliably in this model (Fig. 2A). The dose chosen for the present study is approximately 4 times the calculated ID₅₀ value (7 μg/kg i.t.) in this model (Poppel et al., 1999; Kuss et al., 2002c). Beclomethasone, a well established corticosteroid, was used as reference compound. For this steroid, we selected 1 μg/kg i.t. as the dose to be tested. Indeed, beclomethasone was effective when given 2 and 6 h before challenge.

Similar experiments investigating the time course of action in the same model were published previously for a different PDE4 inhibitor, i.e., roflumilast. With this drug, a prolonged duration of anti-inflammatory action was observed when it was given orally for up to 18 h before allergen challenge (Bundschuh et al., 2001). Roflumilast is currently under investigation in clinical trials for asthma (phase III) and COPD (phase II) using a once-daily treatment schedule.

From the data obtained in this asthma model, AWD 12-281 exerted a very long duration of action when given intratracheally (intrapulmonarily). However, the reasons for the long duration of action of these two drugs may be very different. Although roflumilast has been shown to have a long half-life and also to have an active metabolite (Bundschuh et al., 2001), this is not the case for AWD 12-281. We interpret the results that the long duration of action of our compound is due to persistence of the active compound in the target tissue. Indeed, the half-life of AWD 12-281 after systemic administration in rats is short, and plasma exposure after intratracheal administration is very low (R. Hempel, personal communication). The data indicate that AWD 12-281 may be effective in patients when given once daily.

The most dangerous and life-threatening feature of bronchial asthma is the strong bronchoconstriction that occurs in response to allergen contact. In asthmatics, contact with allergen triggers the mediator release from mast cells (histamine and leukotrienes), consequently leading to the so-called “early phase” bronchoconstriction (within minutes after allergen contact) and contributing to the chronic eosinophilic airway inflammation. To bring about immediate relief and to ensure breathing, inhaled bronchodilators (most importantly β₂ agonists) are used.

Bronchial anaphylaxis in sensitized guinea pigs is a popular model of early, “immediate-type” allergic bronchoconstriction (Andersson, 1980; Payne and De Nucci, 1987). With this model of allergen-induced (early phase) bronchospasm in anesthetized guinea pigs, the antiallergic and bronchodilating properties of new compounds can be assessed. Published data suggest that both PDE3 and PDE4 inhibitors possess moderate bronchodilatory activity in animals, with the former class of compounds being somewhat more effective than the latter (Torphy, 1998). However, this property depends on endogenous catecholamine release (Underwood et al., 1997) and is not observed without adrenergic stimulation. The bronchodilatory activity of the selective PDE4 inhibitors roflumilast and cilomilast has been demonstrated in actively sensitized guinea pigs by oral drug administration (Underwood et al., 1998; Bundschuh et al., 2001). On the basis of these results, it can be expected that the selective PDE4 inhibitor AWD 12-281 should have a bronchodilatory effect against bronchial anaphylaxis by inhaled OVA challenge in sensitized guinea pigs. Indeed, our data show that the compound did show a bronchodilatory effect in this mode if applied intrapulmonarily as a dry powder formulation 1 h before allergen challenge. With the shorter pretreatment time of 30 min, the effect could not be shown. We interpret the data that the effect observed 1 h after i.t. administration is a direct drug effect. Because AWD 12-281 is practically insoluble in water, it may need longer time (1 h) to successfully penetrate the tissue to be active in the bronchial smooth muscle cells. However, this drug characteristic was selected intentionally as part of the optimization process for topical administration.

BHR is a typical feature of bronchial asthma and is characterized by shortness of breath or coughing in response to cold or dusty air or exercise. Therefore, BHR has a major impact on the quality of life of asthma patients. The pathophysiology of BHR is not fully understood; mucosal inflammation, altered neuronal response, and epithelial shedding are known to be involved. BP-2 mice, when actively sensitized to OVA and challenged intranasally, develop bronchial hyperreactivity in response to i.v. methacholine. In addition, an intense eosinophil recruitment into the lung (i.e., an eosinophilic inflammation) is induced. Therefore, this model is well suited to investigate the effects of new drugs on both BHR and lung eosinophilia (Eum et al., 1995). We investigated the effects of repeated drug treatment during the phase of intranasal challenge on BHR and lung eosinophilia. This treatment schedule was selected to suppress the development of the hyperresponsiveness and to avoid direct bronchodilatory drug effects as factors influencing allergically induced BHR. The BHR is assessed by direct measurement of changes in airway resistance in response to i.v. methacholine, a standard procedure to evaluate hyperresponsiveness in asthmatic patients. Using this model, we have been able to show that the repeated treatment with selective PDE4 inhibitors (AWD 12-281 and SB 207499) can reduce BHR and eosinophilic lung inflammation. In contrast, dexamethasone,
although showing a very strong anti-inflammatory effect, had only weak effects on BHR.

COPD is a common, progressive respiratory disease that causes great morbidity and mortality despite treatment. There is evidence for chronic airway inflammation in COPD, which, however (in contrast to asthma), is predominantly a neutrophilic inflammation. Upon degranulation, neutrophilic granulocytes release proteases, oxidizing compounds and chemooattractants, substances that can alter or destroy the physiology of the lung tissue and attract other immune cells (Shenkar and Abraham, 1999). This process is debilitating for the lung, reducing lung flexibility and consequently lung volume. It has been shown that orally active, potent, selective PDE4 inhibitors such as cilomilast or roflumilast, which can suppress the inflammatory reaction, are in vitro (Barnette et al., 1998; Hatzelmann and Schudt, 2001) and in animal models of neutrophilic inflammation (Bundschuh et al., 2001; Spond et al., 2001), are of clinical benefit in patients with COPD (Compton et al., 2001; Leichtl et al., 2002).

Because of the clinical validation of the target PDE4 for the treatment of patients with COPD, we selected LPS-induced neutrophilia in different animal species such as Lewis rats, ferrets, and domestic pigs, as symptom models of inflammation in COPD. Drugs that inhibit neutrophil inflammation in such models are of therapeutic interest (Barnette, 1999; Schmidt et al., 1999; Torphy et al., 1999; Barnes, 2000). AWD 12-281 inhibited potently the LPS-induced lung neutrophilia in BAL fluid, and it was most effective when administered in small doses intratracheally as a dry powder (ID50 of 0.02 μg/kg in rats and 10 μg/kg in ferrets). AWD 12-281 was tested in comparison with the glucocorticoids, which are well known to inhibit acute influx in inflammatory cells after endotoxin shock. After inhaled administration in domestic pigs, AWD 12-281 was shown to be as effective as steroids, albeit at doses about 5 to 10 times higher than those for the glucocorticoid beclomethasone. Intravenous AWD 12-281 at 1 mg/kg i.v. totally suppressed the airway neutrophilia, indicating that the PDE4 inhibitor is as effective as dexamethasone at the dose of 0.28 mg/kg i.v.

Dose Separation of Anti-Inflammatory and Emetogenic Effects

A key problem for the clinical use of PDE4 inhibitors is their emetic potential. The mechanism of emesis induced by these compounds is not fully understood, and there is evidence both for and against a role of the rolipram-binding site, which is a second (high-affinity) binding site for rolipram outside the catalytic site, for and against peripheral effects due to induction of gastric acid secretion and direct effects on the emetic center in the central nervous system. AWD 12-281 has a low affinity for the rolipram-binding site (Hoeffen et al., 1999; Kuss et al., 2002a) and was shown not to induce significant gastric acid secretion (J. I. Szelenyi, unpublished data). In addition, AWD 12-281 had only marginal antidepressant activity in mice after i.p. administration (C. Tober, personal communication), which may be related to low brain penetration. Indeed, in a comparison of the emetic potential and pharmacological effects after systemic administration in ferrets and domestic pigs, the profile shown by AWD 12-281 was better than the profile of other PDE4 inhibitors (Table 1). The therapeutic window (ratio) for AWD 12-281 in ferrets is approximately 4-fold for p.o. and >100 for i.t. administration (emesis/inhibition neutrophilia), respectively. In pigs, the therapeutic window for AWD 12-281 is >9-fold for i.v. and >50 for i.t. administration. Therefore, the emetic dose of AWD 12-281 is clearly separated from the therapeutic dose. After i.t. dosing, the ratio is >10. This view has been supported in toxicological studies in dogs with inhalative administration of AWD 12-281: even the highest technically feasible dose failed to induce emesis.

Our approach for the design of a PDE4 inhibitor with an increased therapeutic window has been the optimization for topical treatment. Based on our interpretation of the data, the topical administration of AWD 12-281 provides a high concentration in the inflamed tissue but the systemic exposure is limited by low oral bioavailability (less than 3%) and extensive glucuronidation. The high doses of AWD 12-281 needed to induce significant anti-inflammatory effects after oral administration are consistent with the pharmacokinetic data (i.e., low oral bioavailability) previously described by Kuss et al. (2002a) and Gasparic et al. (2002). In addition, the compound seems to persist in lung tissue after intratracheal administration, being pharmacologically active for >18 h (Fig. 3). Additionally, AWD 12-281 binds comparatively weakly to the rolipram binding site of the PDE4 isoenzyme (Hoeffen et al., 1999; Kuss et al., 2002a). It also shows a marked binding to plasma proteins. All these factors contribute to the pharmacodynamic and safety profile, i.e., the strong and long-lasting effects after topical administration combined with the excellent tolerability. One problem of inhalative administration may be topical tolerability. However, after intrapulmonary administration of even high doses of AWD 12-281 in rats, no damage of the airway mucosa was observed (Kuss et al., 2002d). Therefore, AWD 12-281 is exceptionally well suited for the topical treatment of bronchial asthma, COPD, and allergic rhinitis. In safety pharmacological and toxicological studies, no negative findings have emerged.

We conclude that AWD 12-281 has a strong anti-inflammatory effect in animal models of asthma and COPD and has a considerably lower emetic potential than rolipram, RP 73401, SB 207499, and roflumilast. AWD 12-281 has been optimized for topical application and is currently under investigation in clinical trials for asthma/COPD and allergic rhinitis.

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References


