British Journal of Pharmacology (2002) 135, 1655–1664

www.nature.com/bjp

Pharmacological profile of PKF242-484 and PKF241-466, novel dual inhibitors of TNF-α converting enzyme and matrix metalloproteinases, in models of airway inflammation

*,[1]Alexandre Trifilieff, [1]Christoph Walker, [1]Thomas Keller, [2]Georg Kottirsch & [3]Ulf Neumann

[1]Novartis Respiratory Research Centre, Horsham, East Sussex; [2]Novartis Pharmaceutical Corporation, Summit, New Jersey, U.S.A. and [3]Novartis Pharma AG, Basel, Switzerland

1 TNF-α converting enzyme (TACE) and matrix metalloproteinases (MMPs) are believed to play a role in various airway inflammatory disorders. Therefore we have tested the effect of two new inhibitors of TACE/MMPs (PKF242-484, PKF241-466) in models of airway inflammation.

2 PKF242-484 and PKF241-466 inhibited purified MMP-1, -2, -3, -9, -13 and rat collagenase at low nanomolar range. Both compounds inhibited the TNF-α release from activated human peripheral blood mononuclear cells with IC_{50} values of 56 ± 28 and 141 ± 100 nM, respectively and had no significant effect on the activation of other human leukocytes, as neither neutrophils and eosinophils oxidative burst nor proliferation or cytokines production by T cells were inhibited *in vitro*.

3 PKF242-484 and PKF241-466 had beneficial effects in two different murine models of acute lung inflammation *in vivo*. The influx of neutrophils and lymphocytes into the airways was reduced 3 and 24 h after intranasal LPS challenge. This was accompanied by reduced levels of myeloperoxidase and elastase activities in the bronchoalveolar lavage. Furthermore, a complete inhibition of TNF-α release into the airways was observed. In addition, PKF242-484 effectively reduced the influx of neutrophils, eosinophils and lymphocytes in a model of acute allergic lung inflammation.

4 PKF242-484 and PKF241-466 are two novel and potent dual inhibitors of TACE and MMPs, which show activity in *in vivo* models of lung inflammation. Such compounds could have beneficial effects in airway inflammatory conditions such as asthma and chronic obstructive pulmonary disease.

British Journal of Pharmacology (2002) **135,** 1655–1664

Keywords: Matrix metalloproteinases; TNF-α converting enzyme; lung; inflammation; *in vivo*

Abbreviations: FCS, foetal calf serum; fMLP, f-Met-Leu-Phe; HSA, human serum albumin; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; TACE, TNF-α converting enzyme; TNF-α, tumour necrosis factor-alpha

Introduction

Chronic obstructive pulmonary disease and asthma are two inflammatory disorders of the airways. Chronic obstructive pulmonary disease is characterized by airway obstruction that is both, slowly progressive and irreversible. This disease encompasses two related conditions; chronic bronchitis and emphysema. Chronic bronchitis is characterized by mucus hypersecretion and an increased risk of chest infections, while the hallmark of emphysema is progressive airflow limitation, largely due to enzymatic destruction of elastin fibres in the lung parenchyma. Both conditions have an inflammatory component which is characterized by the infiltration of neutrophils and macrophages into the lungs (Jeffery, 1998).

Bronchial asthma is a chronic inflammatory disease of the airways, a prominent feature of which is an intense infiltration of inflammatory cells. Of these, the eosinophils are believed to be the main protagonist in inflicting injury to the bronchial mucosa. This damage contributes to bronchial obstruction, hyperreactivity and to the symptoms of asthma (Djukanovic *et al*., 1990).

Tumour necrosis factor-alpha (TNF-α) is produced as a 26 kDa integral membrane protein which is translocated to the cell surface (Kriegler *et al*., 1988). Soluble, 17 kDa TNF-α is released by a membrane enzyme called TNF-α converting enzyme (TACE). Its sequence (Moss *et al*., 1997) indicates that TACE is a member of the ADAM subfamily of Zn-dependent neutral proteinases (Black *et al*., 1997). There is considerable homology in primary and tertiary structure between enzymes from the ADAM and the matrix metalloproteinases (MMP) sub-families (Botos *et al*., 1996). Certain low molecular weight inhibitors of MMP enzymes have been shown to be effective towards TACE and in cellular assays of TNF-α release (Gearing *et al*., 1994). TNF-α is known to cause airway neutrophilia and eosinophilia in various animal models and humans and inhibition of TNF-α is in general associated with an inhibition of the infiltration of proinflammatory cells into the lung tissue (Kips *et al*., 1992; Lukacs *et al*., 1995;

*Author for correspondence at: Novartis Respiratory Research Centre, Wimblehurst Road, Horsham RH12 5AB;
E-mail: alexandre.trifilieff@pharma.novartis.com

Zuany-Amorim *et al*., 1995; Yasui *et al*., 1995; Conroy *et al*., 1995). Significantly, increased levels of TNF-α have been found in sputum of patients with either chronic obstructive pulmonary disease (Keatings *et al*., 1996) or asthma (Shah *et al*., 1995) suggesting that this cytokine could play an important role in regulating both neutrophil and eosinophil trafficking. Thus, blocking the production of TNF-α would be expected to reduce the infiltration of inflammatory cells into the airways of patients with chronic obstructive pulmonary diseases and asthma and thereby, limit pathological changes induced by these cells in the airways.

MMPs are enzymes that have the ability to degrade most of the extracellular matrix proteins. They are expressed at low levels in normal subjects and their activities are tightly regulated by their natural inhibitors, tissue inhibitors of MMPs (Shapiro, 1994). Increasing evidence indicates a key role for at least some of the MMPs in the development of emphysema in chronic obstructive pulmonary diseases patients (Finlay *et al*., 1997; Hautamaki *et al*., 1997; Ohnishi *et al*., 1998) and lung remodelling in asthmatic subjects (Vignola *et al*., 2000). Beside their involvement in tissue degradation and/or remodelling, MMPs also play a crucial role in the trafficking of inflammatory cells *in vitro* (Leppert *et al*., 1995; Delclaux *et al*., 1996; Shipley *et al*., 1996; Okada *et al*., 1997) and may be involved in inflammatory cell migration *in vivo* (Kumagai *et al*., 1999; Shipley *et al*., 1996).

Based on the above, PKF242-484 and PKF241-466, two new dual inhibitors of both the TNF-α convertase enzyme and MMPs, may be predicted to have the potential to target both the inflammatory component and the tissue destruction and/or remodelling characteristically found in both chronic obstructive pulmonary diseases and asthma patients. In this report we have described the anti-inflammatory activity of PKF242-484 and PKF241-466 in animal models of airway inflammation.

Methods

Enzymes assays

In vitro inhibition of human recombinant MMP-1, -9, -13 (obtained from V. Ganu, Novartis Pharmaceuticals Corporation, Summit, NJ, U.S.A.) and -2 (Boehringer Mannheim, Germany) as well as rat collagenase (from J. Jeffrey, Albany, NY, U.S.A.) was measured using the fluorescence-quenched peptide substrate Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (5 μM) and MMP-concentrations of 0.1 nM in 0.1 M Tris-HCl pH 7.5 containing 0.1 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 0.1% PEG6000, at 25°C. Compounds (11 concentrations), enzyme, and buffer were incubated in a black 96-well plate for 1 h. The reaction was started upon the addition of substrate and fluorescence (325/400 nm) was measured for 20 min in a Spectramax Gemini plate reader (Molecular Devices). The rates were fitted to the equation $v = v_o/(1 + [I]/K_i)$, where v and v_o are the rates in presence and absence of inhibitor, respectively, and K_i is the inhibitor constant. The substrate concentration of 5 μM is $<< K_M$ for all MMPs, and no correction for substrate competition is needed. MMP-3 was assayed using Ac-Pro-Leu-Ala(S)-Nvl-Trp-NH_2 and DTNB in 50 mM MES, 10 mM $CaCl_2$ at pH 6.5 (Stein & Izquierdo-Martin, 1994). Inhibitors and enzyme were pre-incubated for 3 h, and reaction was followed at 410 nm in a BioTec Elx808 plate reader for 40 min.

Oxidative burst from human neutrophils and eosinophils

Blood was obtained from normal individual donors. Granulocytes were separated from mononuclear cells by Ficol-hypaque gradient centrifugation. Erythrocytes were lysed by two cycles of hypotonic lysis and the remaining granulocytes were either used as enriched neutrophil preparation (>95% neutrophils) or incubated with anti-CD16 coated immunomagnetic particles to purify eosinophils. Magnetically labelled neutrophils were then depleted by passing the granulocytes through a MACS (magnetic cell separation) column which resulted in a more than 98% pure eosinophil preparation. Both, neutrophils and eosinophils (5×10^4 cells $well^{-1}$ in RPMI 1640 containing 0.1% HSA) were then stimulated with plate-bound human IgG (Sandoglobin coated 96 well microtitre plates, 50 μg $well^{-1}$) or fMLP (Sigma, Poole, U.K., 10 μM) and the ability of the cells to generate superoxide anions during an oxidative burst was measured using a cytochrome C reduction assay (Sigma, Poole, U.K.). The IgG and fMLP induced changes in substrate consumption were monitored over 60 min and results were calculated as percentage of IgG or fMLP induced cytochrome C reduction in the absence of compounds.

Proliferation and cytokine production of human peripheral blood lymphocytes

Mononuclear cells were isolated from the blood of normal individuals by Ficoll-hypaque gradient centrifugation (20 min at $800 \times g$). The interface within the gradient was collected, washed twice in PBS and resuspended in RPMI 1640 supplemented with 10% FCS. Cell density was adjusted to 1×10^6 cell ml^{-1}. For the proliferation assay, 100 μl of the mononuclear cells suspension was placed in 96 well culture plates and 50 μl of either medium or compounds at the indicated concentrations were added. After a 10 min preincubation, cells were stimulated with 50 μl of anti-CD3 monoclonal antibodies (OKT-3, 100 ng ml^{-1}) and then incubated for 42 h at 37°C in a humidified incubator with 5% CO_2. [^{3}H]-Thymidine was added to the culture plates and after 6 h incorporated radioactivity was measured in an automated liquid scintillation counter. For the cytokine measurements, cultures were prepared in the same way as for the proliferation assays. Supernatants were harvested after 20 h of incubation. The levels of IL-5 and IFN-γ were measured by sandwich ELISA. Antibodies used for measuring IL-5 and IFN-γ were purchased from Pharmingen (San Diego, CA, U.S.A.). In all cases, binding of the second antibody was analysed by stepwise incubation with streptavidin-alkaline phosphatase conjugate (Sigma, Poole, U.K.) and 4-nitrophenylphosphate disodium salts (Sigma, Poole, U.K.). Optical density was measured at 405 nm and cytokine concentration was calculated relative to a standard curve plotted from serial dilutions of standard recombinant mouse IL-5 and IFN-γ, respectively. The sensitivity of the cytokine ELISAs was around 10 pg ml^{-1}.

TNF-α production by human peripheral blood mononuclear cells (PBMC)

Mononuclear cells were isolated as above and resuspended in RPMI 1640 supplemented with 10% FCS to a cell density of 10^6 cell ml^{-1}. Cells were stimulated with LPS (10 μg/ml, LPS, *Salmonella Typhosa*) and IFN-γ (50 ng/ml) and supernatants were harvested after 20 h of incubation at 37°c in a humidified incubator with 5% CO_2. Concentration of TNF-α in the supernatants was measured by sandwich ELISA using two monoclonal antibodies recognizing different epitopes of the specific cytokine (mAb357/101-4 and biotinylated 2-179/E11, Novartis Pharma, Switzerland). Binding of the second antibody was analysed by stepwise incubation with streptavidin-alkaline phosphatase conjugate (Mabtech, Stockholm, Sweden) and 4-nitrophenylphosphate disodium salt (Sigma, Poole, U.K.). Optical density was measured at 405 nm and cytokine concentration was calculated by comparison to a standard curve plotted from serial dilutions of standard recombinant human TNF-α.

Animals

Female BALB/c mice or C57BL/6 (4–5 weeks old) were purchased from Harlan (Oxon, U.K.). The animals were housed in plastic cages in an air-conditioned room at 24°C in a 12 h light–dark cycle. Food and water were available *ad libitum*. The studies reported here conformed to the U.K. Animals (scientific procedures) Act of 1986.

LPS-induced accumulation of neutrophils into the lung

BALB/c mice were treated intranasally, under halothane/oxygen/nitrous oxide anaesthesia, with 0.3 mg kg^{-1} of LPS (*Salmonella Typhosa*, Sigma, Poole, U.K.) in 50 μl of PBS or with PBS alone. At either 3 or 24 h following provocation, anaesthesia was induced with pentobarbitone sodium (60 mg kg^{-1}, i.p.).

Ovalbumin-induced lung inflammation

C57BL/6 mice were immunized intraperitoneally with 20 μg of ovalbumin, (grade V, Sigma, Poole, U.K.) in 0.2 ml of Alum (Serva, Heidelberg, Germany) on day 0, and again on day 7. On day 14, animals were exposed, for 20 min, to an aerosol of ovalbumin in PBS (50 mg ml^{-1}) or PBS alone. Twenty-four hours after the challenge, terminal anaesthesia was induced with pentobarbitone sodium (60 mg kg^{-1}, i.p.).

Assessment of lung inflammatory cells and bronchoalveolar lavage soluble mediators measurement

After anaesthesia, the abdominal cavity was opened and the animal exsanguinated by withdrawal of blood from a major blood vessel. The trachea was cannulated and bronchoalveolar lavage was performed by washing four times with 0.3 ml of PBS into the lung *via* the trachea. The fluid was immediately withdrawn and the cell suspension stored on ice. The total cell count was measured and cytospin preparation (Shandon Scientific Ltd, Cheshire, U.K.) prepared. Cells were stained with Dif-Quik (Baxter Dade AG, Dudingen, Switzerland) and a differential count of 200 cells performed using standard morphological criteria. The remaining lavage fluid was centrifuged at $200 \times g$ for 10 min, the supernatant was either used fresh, or aliquoted and stored at −80°C.

Myeloperoxidase activity was measured on fresh bronchoalveolar lavage supernatant using a 96 well plate format colorometric assay. To duplicate 50 μl samples of bronchoalveolar supernatant was added 100 μl of the substrate buffer for 5 min at room temperature (sodium phosphate 50 mM, pH 6.0 containing, 0.5% hexadecyltrimethylammonium bromide, 0.167 nM O-dianiside dihydrochloride and 0.4 mM H_2O_2). The reaction was stopped with 100 μl of 5% sodium azide in distilled water and the OD read at 450 nm. Results were expressed as Unit ml^{-1} using a standard curve established with human leukocyte myeloperoxidase (Sigma, Poole, U.K.).

Elastase activity was measured using a fluorogenic substrate specific for neutrophil elastase, N-methoxysuccinyl-Ala-Ala-Pro-Val-7-amido-4-methyl coumarin (Sigma, Poole, U.K.) (Castillo *et al*., 1979). To 10 μl of bronchoalveolar supernatant was added 90 μl of substrate (1 mM in 0.05 M Tris, 0.5 M NaCl, 0.01 M $CaCl_2$, 10% DMSO, pH 7.5), which was then incubated in the dark at 37°C in white maxisorb fluoronunc plates (Life Technologies, U.K.). Purified human leukocyte elastase (0.1 to 10 ng, Sigma, Poole, U.K.) was used to plot a standard curve. The reaction is continuous and the incubation time varied from 10 min to 4 h, depending on the elastase content in the samples. The cleavage of the substrate was measured using a Spectramax Gemini plate reader (Molecular Devices) set at 370 nm excitation and 460 nm emission. TNF-α and IL-5 levels were measured on stored bronchoalveolar lavage supernatant using commercially available ELISA kits (Genzyme Immunobiologicals, Cambridge, U.K.).

Drug administration

PKF242-484 and PKF241-466 were synthesized by the department of chemistry (Novartis Pharmaceutical Corporation, Summit, NJ, U.S.A.). For the LPS model, mice were treated intranasally, under halothane/oxygen/nitrous oxide anaesthesia, 30 min before and 6 h after the challenge, with compounds dissolved in PBS. Control mice received 50 μl of sterile PBS. In another experiment mice were treated orally, 1 h before the challenge, using 0.1 ml of polyethyleneglycol 300 as vehicle. For the ovalbumin model, mice were treated *via* the intranasal route, as described above, 15 min before and 3 h after the challenge.

Data analysis

Data are expressed as mean ± s.e.mean. Statistical comparisons were performed using a Kruskal Wallis test with Bonferroni correction for multiple comparison and a P value of less than 0.05 was considered significant.

Results

Activities in vitro

The structures of PKF242-484 and PKF241-466 are shown in Figure 1. Both compounds inhibited various human MMP enzymes and rat collagenase activities at low nanomolar

range (Table 1). Both PKF242-484 and PKF241-466 were potent inhibitors of TNF-α release from human PBMC when stimulated *in vitro* with LPS and IFN-γ. PKF242-484 was about three times more potent than PKF241-466 (Table 2). None of the compounds were able to significantly inhibit the *in vitro* activation of neutrophils, eosinophils and T-cells by various stimuli (Table 2).

Effect on LPS-induced lung inflammation

The *in vivo* activity of PKF242-484 and PKF241-466 was first assessed in a model of LPS-induced neutrophil infiltration. At 3 h post-challenge, LPS induced a marked increase in neutrophil and lymphocyte numbers concomitant with a decrease in macrophage numbers in the bronchoalveolar lavage fluid. Intranasal administration of both PKF242-484 and PKF241-466 (30 min before the challenge) induced a dose dependent inhibition of neutrophil and lymphocyte accumulation (Figure 2). Neither compound had an effect on the LPS-induced decrease in macrophage numbers. The LPS-induced TNF-α release in the bronchoalveolar lavage, was fully inhibited by PKF242-484 at the lowest dose used (3 mg kg^{-1}) and thus no dose response could be observed (Figure 3). In agreement with the *in vitro* results, PKF241-466 was less potent than PKF242-484 in inhibiting the LPS-induced TNF-α production (Figure 3).

Because, neutrophil activation parameters (i.e. myeloperoxidase and elastase activities) could not be measured at the 3 h time point (data not shown), a latter time point, 24 h post challenge was assessed. At this time point, the LPS-induced

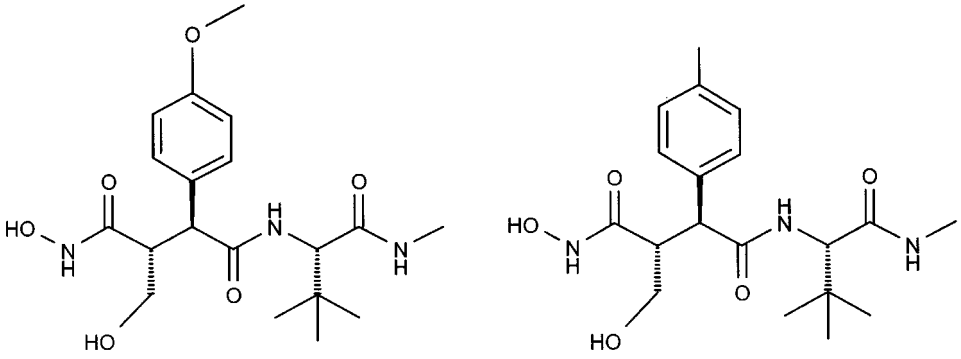

Figure 1 Structure of PKF242-484 [(2S,3R)-N4-((S)-2,2-Dimethyl-1-methylcarbamoyl-propyl)-N1-hydroxy-2-hydroxymethyl-3-(4-methoxy-phenyl)-succinamide) and PKF241-466 [(2S,3R)-N4-((S)-2,2-Dimethyl-1-methylcarbamoyl-propyl)-N1-hydroxy-2-hydroxymethyl-3-phenyl-succinamide].

Table 1 K_i values (nM) for the inhibition of matrix metalloproteinases and TACE by PKF241-466 and PKF242-484

Assay	*PKF241-466* K_i (nM)	*PKF242-484* K_i (nM)
MMP-1 (human collagenase-1)	2.3±0.1 (3)	3.6±0.1 (3)
MMP-2 (human gelatinase A)	0.7±0.1 (3)	0.1±0.01 (3)
MMP-3 (human stromelysin)	25.3±2.4 (3)	0.9±0.1 (3)
MMP-9 (human gelatinase B)	4 (2)	1 (2)
MMP-13 (human collagenase-3)	4.4±0.6 (3)	4.5±0.7 (3)
Rat collagenase	1.7±0.2 (3)	0.04±0.002 (3)

Data are expressed as mean±s.e.mean of the number of experiments indicated in brackets.

Table 2 Effects of PKF241-466 and PKF242-484 on human peripheral blood leukocyte activation

Assay	*PKF241-466* IC_{50} (nM)	*PKF242-484* IC_{50} (nM)
PBMC TNF-α production	141±100 (4)	56.3±28.4 (3)
Neutrophil oxidative burst (IgG)	>5000 (3)	>5000 (3)
Neutrophil oxidative burst (fMLP)	>5000 (3)	1625±487 (3)
Eosinophil oxidative burst (IgG)	>5000 (3)	>5000 (3)
Eosinophil oxidative burst (fMLP)	>5000 (3)	>5000 (3)
T cell IL-5 production	>5000 (2)	>5000 (2)
T cell IFN-γ production	>5000 (2)	>5000 (2)
T cell proliferation	>5000 (2)	>5000 (2)

Data are expressed as mean±s.e.mean of the number of experiments indicated in brackets.

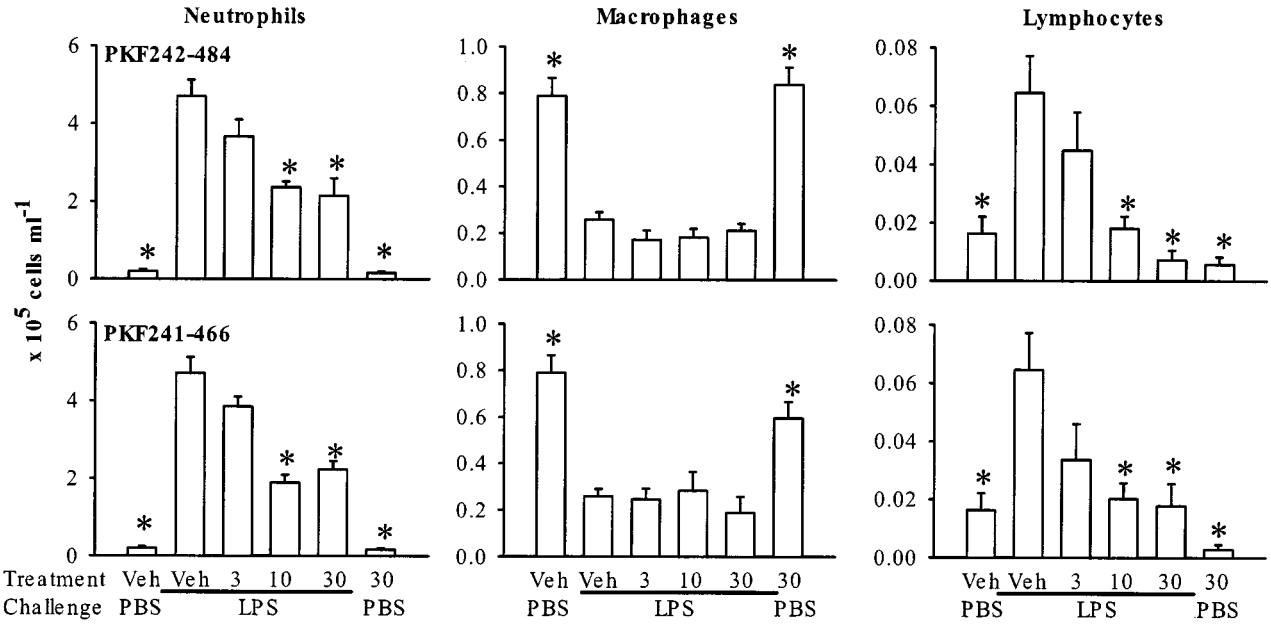

Figure 2 Effect of PKF242-484 and PKF241-466 on bronchoalveolar lavage cellular infiltration 3 h after the LPS challenge. Animals were treated intranasally, 30 min before the LPS or PBS challenge, with vehicle (Veh) or increasing doses of compounds (3 to 30 mg kg^{-1}). Data, from two different experiments, each included 6–8 mice per group, are expressed as mean ± s.e.mean. Significance (*) was determined *versus* vehicle-treated/LPS-challenged group (Veh/LPS).

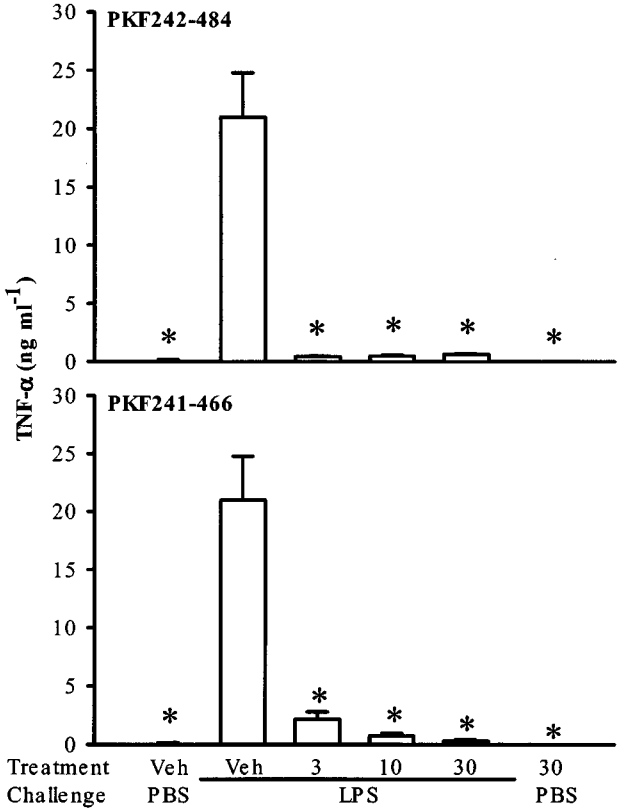

Figure 3 Effect of PKF242-484 and PKF241-466 on bronchoalveolar lavage TNF-α levels 3 h after the LPS challenge. Animals were treated intranasally, 30 min before LPS or PBS challenge, with vehicle (Veh) or increasing doses of compounds (3 to 30 mg kg^{-1}). Data, from two different experiments, each included 6–8 mice per group, are expressed as mean ± s.e.mean. Significance (*) was determined *versus* vehicle-treated/LPS-challenged group (Veh/LPS).

increase in neutrophil and lymphocyte numbers was markedly increased when compared with the 3 h time point. Macrophage numbers were not affected by the LPS challenge. A small, non significant, eosinophilia was also observed (PBS, 0.01 ± 0.01; LPS, 0.03 ± 0.02; $P = 0.14$). Intranasal administration of both PKF242-484 and PKF241-466 (30 min before and 6 h after the challenge) induced a dose-dependent inhibition of neutrophil and lymphocyte accumulation (Figure 4). In parallel with the number of neutrophils, the levels of myeloperoxidase and elastase activities (Figure 5) were also partially inhibited by both compounds. However, this apparent inhibition of *in vivo* cellular activation is probably a reflection of the reduced inflammatory cell number. At the 24 h time point, no detectable levels of TNF-α was observed in the LPS-challenged animals (not shown). When applied without LPS challenge, at a dose of 30 mg kg^{-1}, PKF242-484 and PKF241-466 had no intrinsic effect on any of the parameters measured when examined at both the 3 h (Figures 2 and 3) and 24 h time point (Figures 4 and 5).

To assess whether these compounds were also active when given *via* the oral route, a comparison between oral and intranasal administration of PKF242-484, at 10 mg kg^{-1}, was made in a separate experiment. Groups of six mice were treated orally, 1 h before, and intranasally 30 min before the LPS challenge. Animals were sacrificed at 3 h post LPS challenge. For both routes of administration, PKF242-484 showed a similar profile of inhibition on the cellular infiltration and TNF-α levels (Figure 6).

Effect on ovalbumin-induced lung inflammation

Because PKF242-484 had a slightly better profile than PKF241-466 *in vivo* and *in vitro*, and in order to further characterize our class of components the effect of PKF242-484 in an ovalbumin-driven model of lung allergic inflamma-

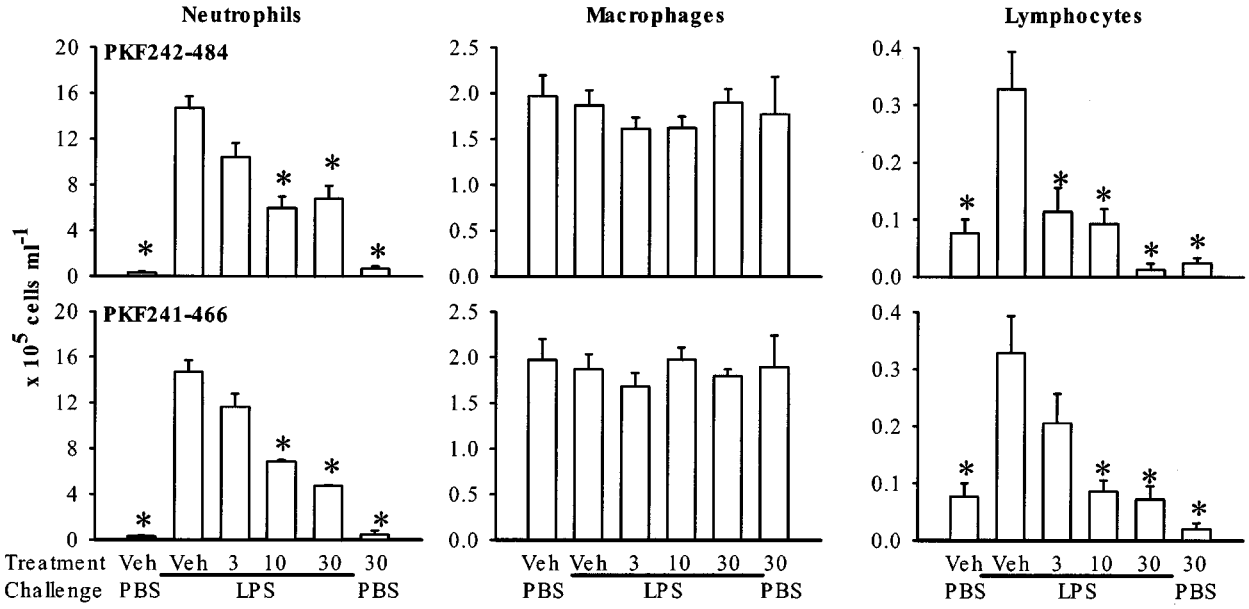

Figure 4 Effect of PKF242-484 and PKF241-466 on bronchoalveolar lavage cellular infiltration 24 h after the LPS challenge. Animals were treated intranasally, 30 min before and 6 h after LPS or PBS challenge, with vehicle (Veh) or increasing doses of compounds (3 to 30 mg kg^{-1}). Data, from two different experiments, each included 6–10 mice per group, are expressed as mean ± s.e.mean. Significance (*) was determined *versus* vehicle-treated/LPS-challenged group (Veh/LPS).

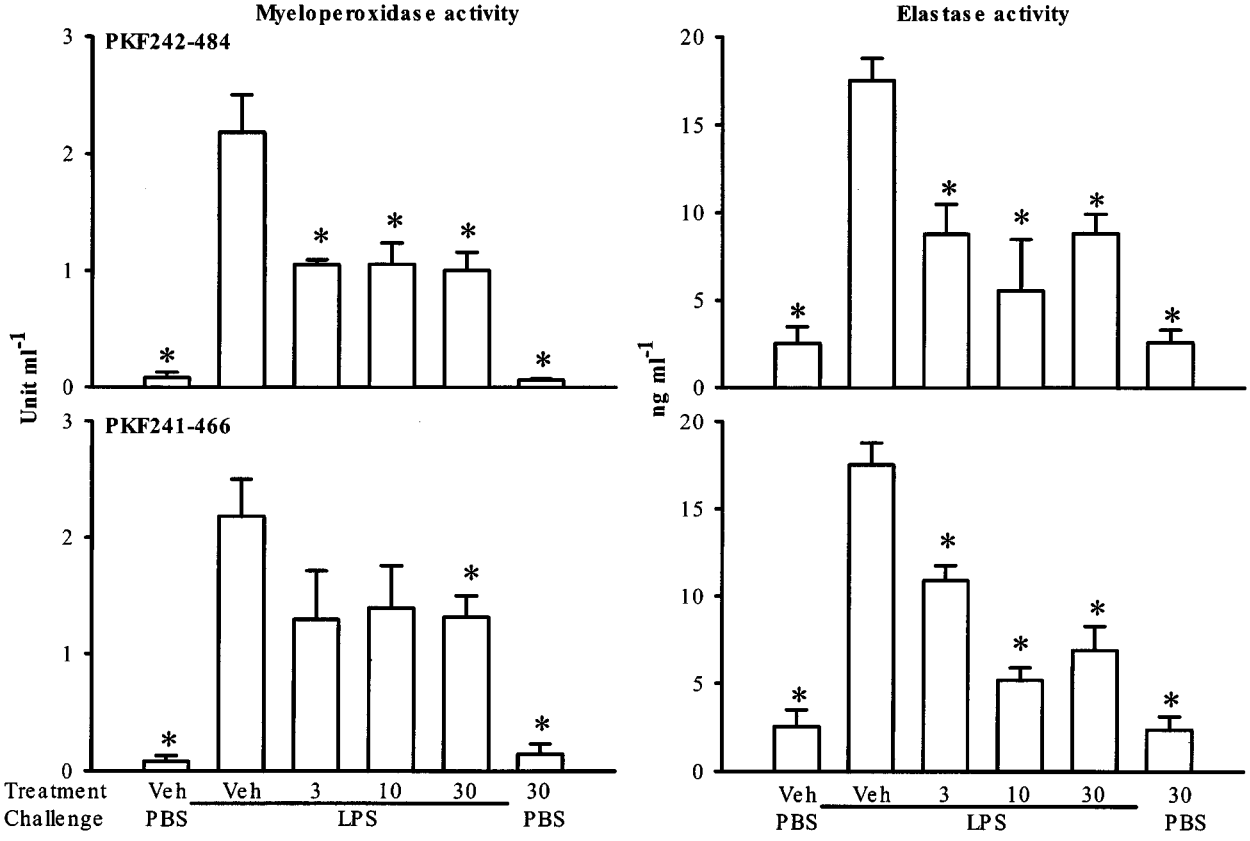

Figure 5 Effect of PKF242-484 and PKF241-466 on bronchoalveolar lavage myeloperoxidase and elastase activities 24 h after the LPS challenge. Animals were treated intranasally, 30 min before and 6 h after LPS challenge or PBS challenge, with vehicle (Veh) or increasing doses of compounds (3 to 30 mg kg^{-1}). Data, from two different experiments, each included 6–10 mice per group, are expressed as mean ± s.e.mean. Significance (*) was determined *versus* vehicle-treated/LPS-challenged group (Veh/LPS).

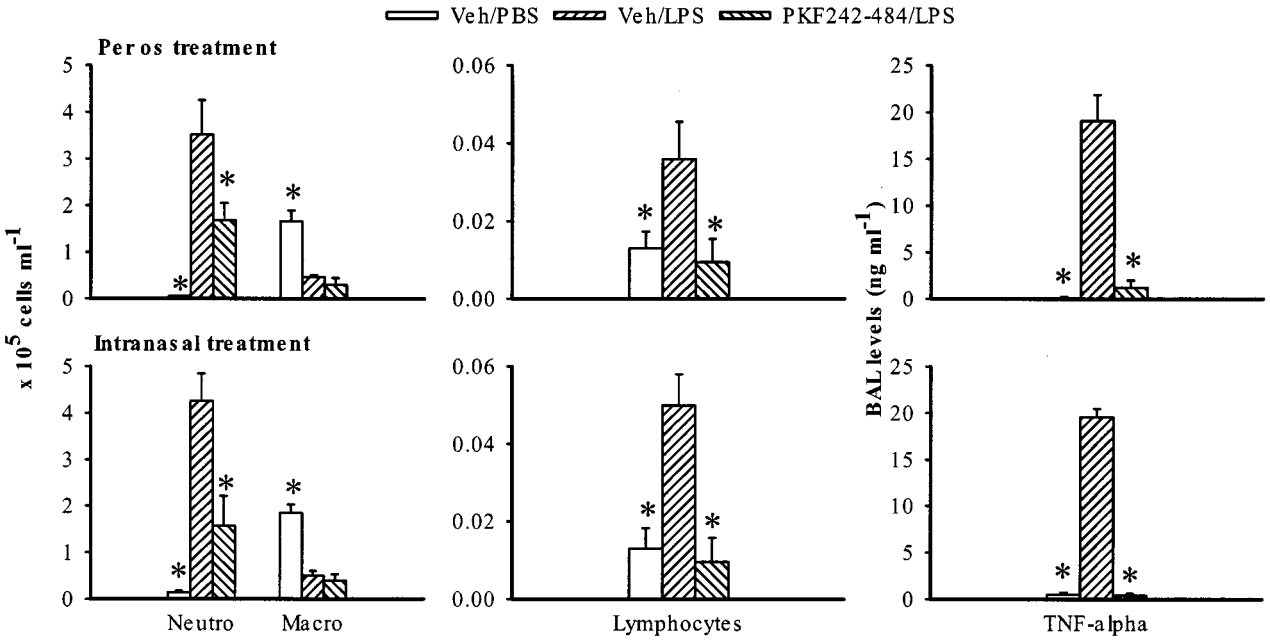

Figure 6 Comparison of oral *versus* intranasal treatment for PKF242-484 (10 mg kg^{-1}) on bronchoalveolar lavage cellular infiltration and TNF-α levels 3 h after the LPS challenge. Animals were treated intranasally 30 min or orally 1 h before LPS or PBS challenge with vehicle (Veh) or 10 mg kg^{-1} of PKF242-484. Data are expressed as mean$\pm$s.e.mean of 6–8 animals per group. Significance (*) was determined *versus* vehicle-treated/LPS-challenged animals (Veh/LPS) in the same treatment group. Neutro: neutrophils; Macro: macrophages.

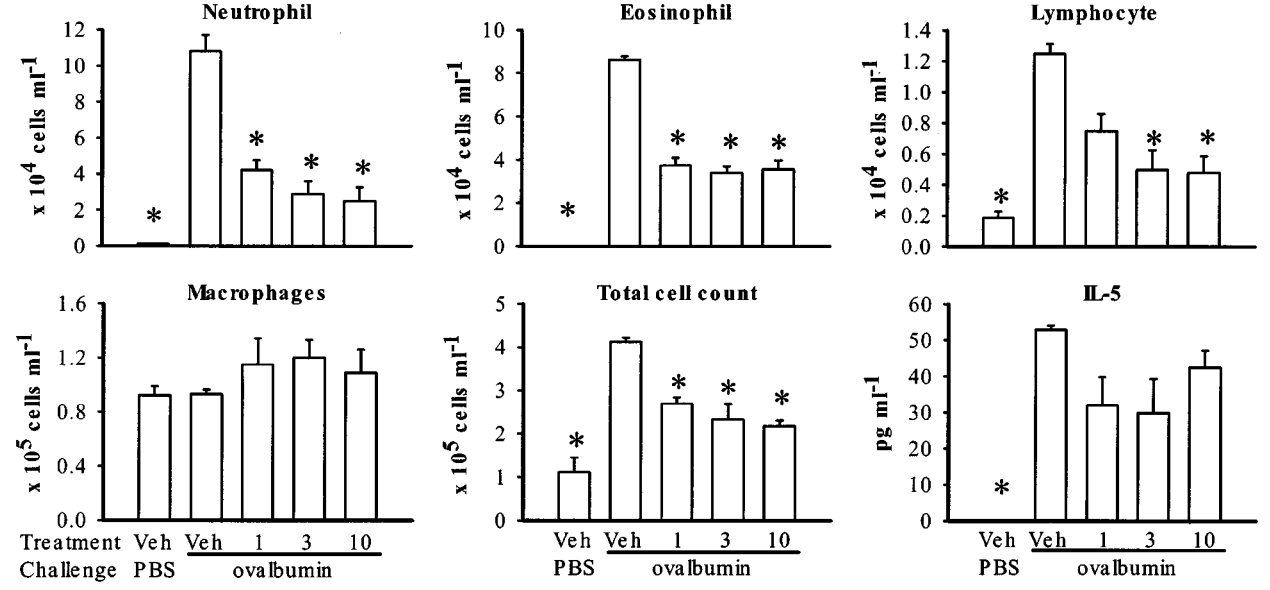

Figure 7 Effect of PKF242-484 on ovalbumin-induced lung inflammation 24 h after the aerosol challenge. Animals were treated intranasally, 30 min before and 6 h after the ovalbumin or PBS challenge, with vehicle (Veh) or increasing doses of PKF242-484 (1 to 10 mg kg^{-1}). Data, from two different experiments, each included 6–8 mice per group, are expressed as mean$\pm$s.e.mean. Significance (*) was determined *versus* vehicle-treated/ovalbumin-challenged animals (Veh/ovalbumin).

tion was studied. In both sensitized and PBS-challenged animals, the majority of the cells recovered in the bronchoalveolar lavage were macrophages and lymphocytes (Figure 7). Twenty-four hours after the ovalbumin challenge, an increase in the number of neutrophils, eosinophils and lymphocytes was observed. Ovalbumin challenge, did not affect macrophage number but did induce an increase in IL-5 levels in the bronchoalveolar lavage (Figure 7). PKF242-484, given intranasally 15 min before and 3 h after the challenge, significantly suppressed the number of neutrophils, eosinophils and lymphocytes present in the bronchoalveolar lavage of these animals and did not affect the number of macrophages (Figure 7). PKF242-484 had no significant effect on bronchoalveolar lavage IL-5 levels (Figure 7).

Discussion

In this study, murine models of lung inflammation were used to demonstrate the ability of PKF242-484 and PKF241-466, two novel dual TACE and MMP inhibitors, to inhibit TNF-α production whilst concomitantly reducing inflammatory cells trafficking into the lung. Although the *in vivo* efficacy for both compounds was studied mainly using intranasal dosing, orally active compounds are highly desirable as therapeutics. To assess whether these compounds were also active when given *via* the oral route, the efficacy of PKF242-484, given both orally or intranasally, in the LPS model was assessed. For both routes of administration, PKF242-484 showed a similar profile of inhibition of lung inflammation, suggesting that this compound has the potential to be an orally active drug.

The *in vivo* activity of PKF242-484 and PKF241-466 was first assessed in a model of LPS-induced neutrophil infiltration. Several lines of evidences indicate that TNF-α should play a significant role in this model. This study and the work of others (Goncalves de Moraes *et al*., 1996; Moreland *et al*., 2001) have shown that, following LPS provocation, a substantial release of TNF-α in the bronchoalveolar lavage is observed. Although these data demonstrated the presence of this cytokine during the early phase of neutrophil recruitment, it does not establish a causal link. Data from studies in mice, demonstrated that lung specific overexpression of TNF-α (Fujita *et al*., 2001) or direct application of TNF-α into the lung (Goncalves de Moraes *et al*., 1996) result in a massive influx of neutrophils. Moreover, it was shown that the lung neutrophil influx observed following LPS application in mice could be partially blocked using neutralizing anti-TNF-α antibodies (Goncalves de Moraes *et al*., 1996) or using TNF receptor p55-deficient mice (Smith *et al*., 1998). In the present study, when PKF242-484 was administered *via* the intranasal route, at a dose which was able to fully inhibit the release of soluble TNF-α in the bronchoalveolar lavage (3 mg kg^{-1}), no effect on the neutrophilic influx was observed. This apparent discrepancy can be explained by the fact that PKF242-484 does not inhibit the synthesis of TNF-α but only its release into its soluble form. It is well documented that membrane-bound TNF-α is functionally active. As such, it can mediate inflammation in experimental models of arthritis (Alexopoulou *et al*., 1997) and hepatitis (Kusters *et al*., 1997) and has also been shown to be increased in number and functionally more active on the surface of macrophages derived from subjects with acute respiratory distress syndrome (Armstrong *et al*., 2000). In summary, published data strongly support the idea that TNF-α is an important mediator that accounts for the large neutrophil influx into the airway following inhalation of bacterial products. Our data, together with that of previously published work (Alexopoulou *et al*., 1997; Kusters *et al*., 1997; Armstrong *et al*., 2000) suggests that the elimination of soluble TNF-α alone is not sufficient to abrogate the inflammatory response to LPS.

It was believed that the inhibition of the LPS-induced neutrophil and lymphocyte increases observed with higher doses (10 and 30 mg kg^{-1}) of both PKF242-484 and PKF241-466 is due to their inhibitory activity on the MMPs. Although we do not present any direct evidence of the involvement of these proteases in our model, LPS has been shown both *in vitro* (Suzuki *et al*., 2000) and *in vivo* (Pagenstecher *et al*., 2000) to induce a dramatic increase the expression of some of the MMPs. In response to LPS, human neutrophils are able to produce gelatinase-B (MMP-9) and this protease has been shown to play a crucial role in neutrophil migration across the basement membrane *in vitro* (Delclaux *et al*., 1996). Similarly, migration of human lymphocytes *in vitro* has also been shown to be highly dependent on the presence of active gelatinases (MMP-2 and -9) (Leppert *et al*., 1995; Xia *et al*., 1996). It should be noted that both PKF242-484 and PKF241-466 did not fully inhibit the LPS-induced neutrophilia (a maximum inhibitory effect of 50% observed at 10 mg kg^{-1} and no further inhibition could be demonstrated at 30 mg kg^{-1}). Whereas, full inhibition was observed for the LPS-induced lymphocyte influx. This suggests that mediators other than MMPs are necessary for neutrophil transmigration following LPS stimulation. Indeed, the neutrophil influx observed following LPS instillation into Golden Syrian hamsters has been shown to be partially dependent on elastase activity (Yasui *et al*., 1995).

Because PKF242-484 had a slightly better profile *in vivo* and *in vitro* and in order to further characterize this class of compounds, it was decided to study the effect of PKF242-484 in an ovalbumin-driven model of lung allergic inflammation. Results obtained showed this compound to be a potent inhibitor of allergen-induced lung inflammatory cell infiltration (e.g. eosinophils, neutrophils and lymphocytes). In a very similar model to the one described in the present study, an increase of the release of MMP-2 and -9 in the bronchoalveolar lavage has been reported following allergen challenge (Kumagai *et al*., 1999). In the same study it was also shown that following treatment with a synthetic inhibitor of MMP (R-14138) or with natural MMP inhibitors such as tissue inhibitor of metalloproteinase-1 and -2, a reduction in the antigen-induced infiltration of lymphocytes and eosinophils into the lungs was observed (Kumagai *et al*., 1999). The role of TNF-α in this model is more controversial. Although it is clear that allergen challenge-induced an early increase in this cytokine (Lukacs *et al*., 1995; Zuany-Amorim *et al*., 1995; Escott *et al*., 2000), the importance of TNF-α in the development of the subsequent inflammatory cell infiltration appears to differ depending on the specific phase of the allergic inflammation. The inhibition of TNF-α activity, using a TNF-α receptor fusion protein (Lukacs *et al*., 1995; Renzetti *et al*., 1996) or a neutralizing anti-TNF-α antibody (Zuany-Amorim *et al*., 1995), was reported to reduce eosinophil recruitment into the lung following a single allergen challenge. However, if a multiple challenge protocol is used, TNF-α neutralization does not have a significant effect on the extent of lung inflammation (Hessel *et al*., 1997; Rudmann *et al*., 2000). Similar conclusions were drawn using mice deficient in TNF-α receptors (Rudmann *et al*., 2000). These published data, suggest that neutralization of TNF-α activity only transiently abrogates the inflammatory response following allergen challenge. In our model, which used a single allergen challenge, it is therefore difficult to distinguish the effects of PKF242-484 due to its inhibitory activity on TACE or the MMPs. The most likely explanation is that PKF242-484 inhibits the inflammatory reaction by acting on both families of enzymes. Cytokines of the Thelper 2 type, such as IL-5, are believed to play a central role in the induction of allergic airway inflammation (Anderson & Coyle, 1994). Interestingly, in our model, the allergen-induced

increase in bronchoalveolar lavage IL-5 levels was not affected by treatment with PKF242-484, whereas the inflammatory cell infiltration was significantly reduced. These results are in accordance with the *in vitro* data obtained following T-cells activation, that the inhibitory action of PKF242-484 on inflammatory cell infiltration was not due to a down regulation of IL-5.

One concern in using dual TACE/MMP inhibitors in the clinic is their possible pro-inflammatory properties. Indeed, *in vitro*, this class of compounds have been shown to decrease the ability of stimulated cells to shed various pro-inflammatory membrane bound proteins such as IL-6 receptor (Mullberg *et al*., 1995), IL-1 decoy receptor (Orlando *et al*., 1997) and L-selectin (Feehan *et al*., 1996). In addition, upon activation and when compared with wild type cells, TACE-deficient mice cells showed an increase in surface TNF-α expression (Black *et al*., 1997) and failed to shed the type II TNF-α receptor (Peschon *et al*., 1998). These observations, have been confirmed using synthetic inhibitors *in vitro* (Mohler *et al*., 1994; McGeehan *et al*., 1994; Williams *et al*., 1996). However, in contrast to these experimental situations, in humans, undesired proinflammatory effects such as enhanced expression of surface TNF-α or surface TNF-α receptor were not observed following a single dose treatment with a TACE/MMP inhibitor (Dekkers *et al*., 1999). Nevertheless, since TACE/MMP inhibitors are likely to be used in the treatment of various inflammatory diseases in the future, it is important to fully address their possible proinflammatory properties before this class of compound is tested in clinical setting.

In summary, we have demonstrated preclinical *in vitro* and *in vivo* evidence to suggest that PKF242-484 and PKF241-466, the novel combined TACE and MMP inhibitors, may have potential for the treatment of inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease.

References

ALEXOPOULOU, L., PASPARAKIS, M. & KOLLIAS, G. (1997). A murine transmembrane tumor necrosis factor (TNF) transgene induces arthritis by cooperative p55/p75 TNF receptor signaling. *Eur. J. Immunol.,* **27,** 2588 – 2592.

ANDERSON, G.P. & COYLE, A.J. (1994). TH2 and 'TH2-like' cells in allergy and asthma: pharmacological perspectives. *Trends Pharmacol. Sci.,* **15,** 324 – 332.

ARMSTRONG, L., THICKETT, D.R., CHRISTIE, S.J., KENDALL, H. & MILLAR, A.B. (2000). Increased Expression of Functionally Active Membrane-Associated Tumor Necrosis Factor in Acute Respiratory Distress Sydnrome. *Am. J. Respir. Cell Mol. Biol.,* **22,** 68 – 74.

BLACK, R.A., RAUCH, C.T., KOZLOSKY, C.J., PESCHON, J.J., SLACK, J.L., WOLFSON, M.F., CASTNER, B.J., STOCKING, K.L., REDDY, P., SRINIVASAN, S., NELSON, N., BOLANI, N., SCHOOLEY, K.A., GERHART, M., DAVIS, R., FITZNER, J.N., JOHNSON, R.S., PAXTON, R.J., MARCH, C.J. & CERRETTI, D.P. (1997). A metalloproteinase disintegrin that release tumor-necrosis factor-α from cells. *Nature,* **385,** 729 – 733.

BOTOS, I., SCAPOZZA, L., ZHANG, D., LIOTTA, L.A. & MEYER, E.F. (1996). Batimastat, a potent matrix metalloproteinase inhibitor, exhibits an unexpected mode of binding. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 2749 – 2754.

CASTILLO, M.J., NAKAJIMA, K., ZIMMERMAN, M. & POWERS, J.C. (1979). Sensitive substrates for human leukocyte and porcine pancreatic elastase: a study of the merits of various chromophoric and fluorogenic leaving groups in assays for serine proteases. *Anal. Biochem.,* **99,** 53 – 64.

CONROY, D.M., FRANCISCHI, J.N. & SIROIS, P. (1995). Effect of tumor necrosis factor receptor binding protein on cell infiltration induced by lipopolysaccharide and Sephadex beads in guinea pig lung. *Inflammation,* **19,** 233 – 243.

DEKKERS, P.E., LAUW, F.N., TEN HOVE, T., TE, V.A., LUMLEY, P., BECHERER, D., VAN DEVENTER, S.J. & VAN DER POLL, T. (1999). The effect of a metalloproteinase inhibitor (GI5402) on tumor necrosis factor-alpha (TNF-alpha) and TNF-alpha receptors during human endotoxemia. *Blood,* **94,** 2252 – 2258.

DELCLAUX, C., DELACOURT, C., D'ORTHO, M.P., BOYER, V., LAFUMA, C. & HARF, A. (1996). Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. *Am. J. Respir. Cell Mol. Biol.,* **14,** 288 – 295.

DJUKANOVIC, R., ROCHE, W.R., WILSON, J.W., BEASLEY, C.R., TWENTYMAN, O.P., HOWARTH, R.H. & HOLGATE, S.T. (1990). Mucosal inflammation in asthma. *Am. Rev. Respir. Dis.,* **142,** 434 – 457.

ESCOTT, K.J., BELVISI, M.G., BIRRELL, M.A., WEBBER, S.E., FOSTER, M.L. & SARGENT, C.A. (2000). Effect of the p38 kinase inhibitor, SB 203580, on allergic airway inflammation in the rat. *Br. J. Pharmacol.,* **131,** 173 – 176.

FEEHAN, C., DARLAK, K., KAHN, J., WALCHECK, B., SPATOLA, A.F. & KISHIMOTO, T.K. (1996). Shedding of the Lymphocyte L-Selectin Adhesion Molecule Is Inhibited by a Hydroxamic Acid-based Protease Inhibitor. *J. Biol. Chem.,* **271,** 7019 – 7024.

FINLAY, G.A., RUSSELL, K.J., McMAHON, K.J., D'ARCY, E.M., MASTERSON, J.B., FITZGERALD, M.X. & O'CONNOR, C.M. (1997). Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients. *Thorax,* **52,** 502 – 506.

FUJITA, M., SHANNON, J.M., IRVIN, C.G., FAGAN, K., COOL, C., AUGUSTIN, A. & MASON, R. (2001). Overexpression of tumor necrosis factor-alpha produces an increase in lung volumes and pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **280,** L39 – L49.

GEARING, A.J., BECKETT, P., CHRISTODOULOU, M., CHURCHILL, M., CLEMENTS, J., DAVIDSON, A.H., DRUMMOND, A.H., GALLOWAY, W.A., GILBERT, R. & GORDON, J.L. (1994). Processing of tumour necrosis factor-alpha precursor by metalloproteinases. *Nature,* **370,** 555 – 557.

GONCALVES DE MORAES, V., VARGAFTIG, B., LEFORT, J., MEAGER, A. & CHIGNARD, M. (1996). Effect of cyclo-oxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. *Br. J. Pharmacol.,* **117,** 1792 – 1796.

HAUTAMAKI, R.D., KOBAYASHI, D.K., SENIOR, R.M. & SHAPIRO, S.D. (1997). Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science,* **277,** 2002 – 2004.

HESSEL, E.M., VAN OOSTERHOUT, A.J., VAN, I.A., VAN ESCH, B., HOFMAN, G., VAN LOVEREN, H., SAVELKOUL, H.F. & NIJKAMP, F.P. (1997). Development of airway hyperresponsiveness is dependent on interferon-gamma and independent of eosinophil infiltration. *Am. J. Respir. Cell Mol. Biol.,* **16,** 325 – 334.

JEFFERY, P.K. (1998). Structural and inflammatory changes in COPD: a comparison with asthma. *Thorax,* **53,** 129 – 136.

KEATINGS, V.M., COLLINS, P.D., SCOTT, D.M. & BARNES, P.J. (1996). Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am. J. Respir. Crit. Care Med.,* **153,** 530 – 534.

KIPS, J.C., TAVERNIER, J. & PAUWELS, R.A. (1992). Tumor necrosis factor causes bronchial hyperresponsiveness in rats. *Am. Rev. Respir. Dis.,* **145,** 332 – 336.

KRIEGLER, M., PEREZ, C., DEFAY, K., ALBERT, I. & LU, S.D. (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell,* **53,** 45 – 53.

KUMAGAI, K., OHNO, I., OKADA, S., OHKAWARA, Y., SUZUKI, K., SHINYA, T., NAGASE, H., IWATA, K. & SHIRATO, K. (1999). Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. *J. Immunol.,* **162,** 4212 – 4219.

KUSTERS, S., TIEGS, G., ALEXOPOULOU, L., PASPARAKIS, M., DOUNI, E., KUNSTLE, G., BLUETHMANN, H., WENDEL, A., PFIZENMAIER, K., KOLLIAS, G. & GRELL, M. (1997). In vivo evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experiental hepatitis. *Eur. J. Immunol.,* **27,** 2870 – 2875.

LEPPERT, D., WAUBANT, E., GALARDY, R., BUNNETT, N.W. & HAUSER, S.L. (1995). T cell gelatinases mediate basement membrane transmigration in vitro. *J. Immunol.,* **154,** 4379 – 4389.

LUKACS, N.W., STRIETER, R.M., CHENSUE, S.W., WIDMER, M. & KUNKEL, S.L. (1995). TNF-alpha mediates recruitment of neutrophils and eosinophils during airway inflammation. *J. Immunol.,* **154,** 5411 – 5417.

MCGEEHAN, G.M., BECHERER, J.D., BAST, R.C.J., BOYER, C.M., CHAMPION, B., CONNOLLY, K.M., CONWAY, J.G., FURDON, P., KARP, S. & KIDAO, S. (1994). Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. *Nature,* **370,** 558 – 561.

MOHLER, K.M.S.P.R., FITZNER, J.N., CERRETTI, D.P., ALDERSON, M., KERWAR, S.S., TORRANCE, D.S., OTTEN-EVANS, C., GREENSTREET, T., WEERAWARNA, K., KRONHEIM, S.R., PETERSEN, M., GERHART, M., KOZLOSKY, C.J., MARCH, C.J. & BLACK, R.A. (1994). Protection against a lethal dose of endotoxin by an inhibitor of tumor necrosis factor processing. *Nature,* **370,** 218 – 220.

MORELAND, J., FUHRMAN, R., WOHLFORD-LENANE, C., QUINN, T., BENDA, E., PRUESSNER, J. & SCHWARTZ, D.A. (2001). TNF-alpha and IL-1beta are not essential to the inflammatory response in LPS-induced airway disease. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **280,** L173 – L180.

MOSS, M.L., JIN, S.-L.C., MILLA, M.E., BURKHART, W., CARTER, H.L., CHEN, W.J., CLAY, W.C., DIDSBURY, J.R., HASSIER, D., HOFFMAN, C.R., KOST, T.A., LAMBERT, M.H., LEESNITZER, M.A., MCCAULEY, P., MCGEELHAN, G., MITCHELL, J., MOYER, M., PAHEL, G., ROCQUE, W., OVERTON, L.K., SCHOENEN, F., SEATON, T., SU, J.-L., WARNER, J., WILLARD, D. & BECHERER, D. (1997). Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α. *Nature,* **385,** 733 – 736.

MULLBERG, J., DURIE, F.H., OTTEN-EVANS, C., ALDERSON, M.R., ROSE-JOHN, S., COSMAN, D., BLACK, R.A. & MOHLER, K.M. (1995). A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. *J. Immunol.,* **155,** 5198 – 5205.

OHNISHI, K., TAKAGI, M., KUROKAWA, Y., SATOMI, S. & KONTTINEN, Y.T. (1998). Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. *Lab. Invest.,* **78,** 1077 – 1087.

OKADA, S., KITA, H., GEORGE, T.J., GLEICH, G.J. & LEIFERMAN, K.M. (1997). Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am. J. Respir. Cell Mol. Biol.,* **17,** 519 – 528.

ORLANDO, S., SIRONI, M., BIANCHI, G., DRUMMOND, A.H., BORASCHI, D., YABES, D. & MANTOVANI, A. (1997). Role of Metalloproteases in the Release of the IL-1 type II Decoy Receptor. *J. Biol. Chem.,* **272,** 31764 – 31769.

PAGENSTECHER, A., STALDER, A.K., KINCAID, C.L., VOLK, B. & CAMPBELL, I.L. (2000). Regulation of matrix metalloproteinases and their inhibitor genes in lipopolysaccharide-induced endotoxemia in mice. *Am. J. Pathol.,* **157,** 197 – 210.

PESCHON, J.J., SLACK, J.L., REDDY, P., STOCKING, K.L., SUNNARBORG, S.W., LEE, D.C., RUSSELL, W.E., CASTNER, B.J., JOHNSON, R.S., FITZNER, J.N., BOYCE, R.W., NELSON, N., KOZLOSKY, C.J., WOLFSON, M.F., RAUCH, C.T., CERRETTI, D.P., PAXTON, R.J., MARCH, C.J. & BLACK, R.A. (1998). An essential role for ectodomain shedding in mammalian development. *Science,* **282,** 1281 – 1284.

RENZETTI, L.M., PACIOREK, P.M., TANNU, S.A., RINALDI, N.C., TOCKER, J.E., WASSERMAN, M.A. & GATER, P.R. (1996). Pharmacological evidence for tumor necrosis factor as a mediator of allergic inflammation in the airways. *J. Pharmacol. Exp. Ther.,* **278,** 847 – 853.

RUDMANN, D.G., MOORE, M.W., TEPPER, J.S., ALDRICH, M.C., PFEIFFER, J.W., HOGENESCH, H. & TUMAS, D.B. (2000). Modulation of allergic inflammation in mice deficient in TNF receptors. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **279,** L1047 – L1057.

SHAH, A., CHURCH, M.K. & HOLGATE, S.T. (1995). Tumour necrosis factor alpha: a potential mediator of asthma. *Clin. Exp. Allergy,* **25,** 1038 – 1044.

SHAPIRO, S.D. (1994). Elastolytic metalloproteinases produced by human mononuclear phagocytes. Potential roles in destructive lung disease. *Am. J. Respir. Crit. Care Med.,* **150,** S160 – S164.

SHIPLEY, J.M., WESSELSCHMIDT, R.L., KOBAYASHI, D.K., LEY, T.J. & SHAPIRO, S.D. (1996). Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 3942 – 3946.

SMITH, S., SKERRETT, S.J., CHI, E.Y., JONAS, M., MOHLER, K. & WILSON, C.B. (1998). The locus of tumor necrosis factor-alpha action in lung inflammation. *Am. J. Respir. Cell. Mol. Biol.,* **19,** 881 – 891.

STEIN, R.L. & IZQUIERDO-MARTIN, M. (1994). Thioester hydrolysis by matrix metalloproteinases. *Arch. Biochem. Biophys.,* **308,** 274 – 277.

SUZUKI, T., HASHIMOTO, S., TOYODA, N., NAGAI, S., YAMAZAKI, N., DONG, H.Y., SAKAI, J., YAMASHITA, T., NUKIWA, T. & MATSUSHIMA, K. (2000). Comprehensive gene expression profile of LPS-stimulated human monocytes by SAGE. *Blood,* **96,** 2584 – 2591.

VIGNOLA, A.M., KIPS, J. & BOUSQUET, J. (2000). Tissue remodeling as a feature of persistent asthma. *J. Allergy Clin. Immunol.,* **105,** 1041 – 1053.

WILLIAMS, L.M., GIBBONS, D.L., GEARING, A., MAINI, R.M., FELDMANN, M. & BRENNAN, F.M. (1996). Paradoxical effects of a synthetic metalloproteinase inhibitor that blocks both p55 and p75 TNF receptor shedding and TNFa processing in RA synovial membrane cell cultures. *J. Clin. Invest.,* **97,** 2833 – 2841.

XIA, M., LEPPERT, D., HAUSER, S.L., SREEDHARAN, S.P., NELSON, P.J., KRENSKY, A.M. & GOETZL, E.J. (1996). Stimulus specificity of matrix metalloproteinase dependence of human T cell migration through a model basement membrane. *J. Immunol.,* **156,** 160 – 167.

YASUI, S., NAGAI, A., AOSHIBA, K., OZAWA, Y., KAKUTA, Y. & KONNO, K. (1995). A specific neutrophil elastase inhibitor (ONO-5046.Na) attenuates LPS-induced acute lung inflammation in the hamster. *Eur. Respir. J.,* **8,** 1293 – 1299.

ZUANY-AMORIM, C., HAILE, S., LEDUC, D., DUMAREY, C., HUERRE, M., VARGAFTIG, B.B. & PRETOLANI, M. (1995). Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J. Clin. Invest.,* **95,** 2644 – 2651.

(Received October 31, 2001
Revised January 15, 2002
Accepted January 16, 2002)