Anti-Inflammatory Effects of the Novel Phosphodiesterase IV Inhibitor RP 73401

J.-A.		Karlsson
J.		Souness
S.		Webber
K.		Pollock
D.		Raeburn

Rhône-Poulenc Rorer Ltd., Dagenham Research Centre, Dagenham, UK

Key Words

Human
Guinea pig
Eosinophil
Neutrophil
Monocyte
Lymphocyte
Tumor necrosis factor- α
Interleukin-2

Correspondence to: Dr. Jan-Anders Karlsson, Dagenham Research Centre, Rhône-Poulenc Rorer Ltd., Rainham Road South, Dagenham Essex RM10 7XS (UK)

At least 7 different cyclic nucleotide phosphodiesterase (PDE) isoenzymes have been identified on the basis of their functional characteristics such as substrate specificity and susceptibility to selective inhibitors. cAMP-specific PDE (type IV) is the major isoenzyme in many inflammatory cells where it is involved in suppression of activation and mediator release. Human airway smooth muscle contains at least five PDE isoenzyme families, but PDE III and PDE IV are functionally the most important as modulators of contractility [1]. We have now characterized the PDE inhibitory, anti-inflammatory and bronchodilator effects of RP 73401 (3-cyclopentyloxy-N-(3,5-dichloro-4-pyridyl)-4-methoxy benzamide), a novel, potent PDE IV inhibitor [2].

PDEs I, III, IV and V were isolated from pig aorta, PDEs II and IV from bovine trachea, and PDE IV from guinea pig peritoneal eosinophils [3]. Human peripheral blood granulocytes and guinea pig peritoneal eosinophils were isolated by density gradient centrifugation. Superoxide anion was measured as the superoxide – dismutase – inhibitable reduction of 7-iodonitrotetrazolium violet. Eosinophil cationic protein (ECP), major basic protein (MBP), neutrophil elas-

tase and cAMP were quantified by RIA. Antigen-aerosol-induced bronchospasm was measured as the increase in specific airway resistance (sRaw) in ovalbumin (OA)-sensitized guinea pigs. Inflammatory cells in bronchoalveolar lavage (BAL) fluid 24 h after challenge were enumerated. In separate experiments, the effects of RP 73401 on platelet activating factor (PAF) [intravenous (i.v) infusion]-induced airway hyperresponsiveness to bombesin and intratracheal (i.t.) PAF-induced microvascular leakage (accumulation of FITC-dextran in tracheobronchial tissue and

BAL fluid) were examined in naive guinea pigs. IC₅₀ values with the SE or 95% confidence intervals are reported.

RP 73401 selectively inhibited PDE IV from guinea pig eosinophils and pig aorta with IC₅₀ values of 0.8 ± 0.5 and 1.0 ± 0.1 μ M, respectively. It was at least 19,000-fold less potent against PDEs I, II, III and V. RP 73401 was considerably more potent than rolipram which inhibited these enzyme preparations with IC₅₀ values of 230 ± 20 and $3,000 \pm 500$ nM. Solubilization or vanadate/glutathione treatment of the guinea pig eosinophil membrane-bound PDE IV increased the potency of rolipram about 10-fold, but did not

© 1995 S.Karger AG, Basel 1018-2438/95/1073-0425 \$8.00/0

affect the potency of RP 73401. In the presence of isoprenaline, RP 73401 increased cAMP levels in intact guinea pig eosinophils.

Challenge of guinea pig eosinophils with leukotriene B₄ produced superoxide generation and release of ECP and MBP. Pretreatment with RP 73401 concentration dependently inhibited mediator release with IC₅₀ values of 30 (10-50) nM, 8.6 (8.2-9.0) nM and 110 (100-120) nM, respectively. FMLP-induced superoxide production and ECP (eosinophils) release from human granulocytes was inhibited by RP 73401 [IC₅₀ 0.27 (0.14-0.40) and 8.1 (7.7-8.5) nM respectively]. Elastase (neutrophils) release was also suppressed [IC₅₀ 6.4 (6.1-6.7) μ M].

Rolipram was 15- to 350 fold less potent than RP 73401 on ECP and elastase release from human granulocytes, but only 3- to 6-fold less potent on superoxide production.

Lipopolysaccharide stimulated tumor necrosis factor- α (TNF- α) release from human blood monocytes was inhibited by RP 73401 [IC₅₀ 4.2 ± 0.3 nM] and so was Staphylococcus enterotoxin-A-induced interleukin-2 release from mouse splenocytes (IC₅₀ 0.2/1.3 nM).

In anesthetized guinea pigs, i.t. administered RP 73401 inhibited antigen-induced bronchospasm (ED₅₀ 7 ± 1 μ g) as well as total cells (3.9 ± 0.8 μ g) and eosinophils (3.2 ± 0.7 μ g) in BAL fluid 24 h later. RP 73401 (i.v.) inhibited PAF-induced airway hyperresponsiveness (ED₅₀ 0.09 ± 0.03 μ g) and i.t. PAF-induced microvascular leakage into tracheo-bronchial tissue and BAL fluid (91 ± 5 and $55 \pm 20\%$ inhibition, respectively, at 25 μ g/kg i.t.).

RP 73401 is a very potent and selective inhibitor of cAMP hydrolysis. It inhibits PDE IV with low nanomolar potency and displays considerable selectivity over PDEs I, II, III and V. Rolipram, a standard PDE IV inhibitor, was considerably less potent and isoenzyme selective. PDE IV is the predominant isoenzyme regulating cAMP levels and (consequently) function in a wide range of inflammatory cells, including granulocytes and monocytes/macrophages, as confirmed by the present in vitro experiments. The number of activated eosinophils is elevated in the asthmatic airway and eosinophil cytotoxic proteins may contribute to pathological changes in the respiratory mucosa and consequently to the increase in airway hyperresponsiveness. Interestingly, RP 73401 inhibited antigen-induced accumulation of eosinophils in guinea pig airways and in vitro mediator release from this cell type, and also PAF-induced airway hyperresponsiveness. Inhibition of TNF- α and IL-2 release could have contributed to reduced eosinophil numbers in BAL through inhibition of adhesion molecule expression on endothelium and eosinophil chemotaxis, respectively. The potent inhibitory effects of RP 73401 on the synthesis and secretion of cytokines and mediators, together with the data on rolipram, indicate the importance of the type IV PDE in regulating cell activation. Indeed, RP 73401 inhibited antigen-induced bronchospasm, probably both by a direct relaxant effect and by suppression of mediator release, cell infiltration and microvascular leakage.

Taken together, in vitro enzyme data indicate that RP 73401 differs from rolipram in that it interacts in a novel way with the PDE IV isoenzyme. RP 73401 potently suppresses inflammatory cell activity and in vivo it has pronounced anti-inflammatory effects in the tracheobronchial tree. RP 73401 is being developed for the treatment of airway inflammation and asthma.

References

- 1 DeBoer J, Philpott AJ, Van Amsterdam RGM, Shahid M, Zaagsma J, Nicholson CD: Human bronchial cyclic nucleotide phosphodiesterase isoenzymes: Biochemical and pharmacological analysis using selective inhibitors. *BrJ Pharmacol* 1992;106:1028-1034.
- Ashton MJ, Cook DC, Fenton G, Karlsson 1A, Palfreyman MN, Raeburn D, Ratcliffe AJ, Souness JE, Thurairaitnam S, Vicker N: Selective type IV phosphodiesterase inhibitors as anti-asthmatic agents: The synthesis and biological activities of 3-cyclopentyloxy-4-methoxybenz-amides and analogues. *J Med Chem* 1994;37: 1696-1703.
- Souness JE, Villamil ME, Scott LC, Tomkinson A, Giembycz MA, Raeburn D: Possible role of cyclic AMP phosphodiesterases in the actions of ibudilast on eosinophil thromboxane generation and airways smooth muscle tone. *Br J Pharmacol* 1994;111:1081-1088.

426

Karlsson/Souness/Webber/Pollock/Raeburn
PDE IV Inhibition and Airway Inflammation