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Short communication

Reduction of matrix metalloproteinase-9 activity by the selective phosphodiesterase 4 inhibitor, RP 73-401 in sensitized mice

Chantal Belleguic [a,b], Marianne Corbel [a], Noëlla Germain [a], Elisabeth Boichot [a], Philippe Delaval [b], Vincent Lagente [a,*]

[a] *INSERM U 456, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes 1, 2, Avenue du Prof. Léon Bernard, 35043 Rennes cedex, France*

[b] *Service de Pneumologie, Hôpital de Pontchaillou, CHR de Rennes, 35000 Rennes, France*

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Abstract

Matrix metalloproteinases are particularly potent in degrading basement membrane collagen and other extracellular matrix components. We have investigated the effects of a selective phosphodiesterase 4 inhibitor, RP 73-401 [*N*-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide], on gelatinase (matrix metalloproteinase-2 and matrix metalloproteinase-9) activity in ovalbumin-sensitized and -challenged mice. Twenty-four hours after the last challenge, matrix metalloproteinase activity was evaluated in the bronchoalveolar lavage fluids by a zymography technique, and a significant increase in matrix metalloproteinase-9, but not matrix metalloproteinase-2, activity was noted. When administered orally (0.3–3 mg/kg) 1 h before each ovalbumin challenge, the selective phosphodiesterase 4 inhibitor, RP 73-401, significantly reduced this increased matrix metalloproteinase-9 activity in bronchoalveolar lavage fluids. Our data suggest that RP 73-401 may modulate tissue remodelling associated with lung inflammatory processes including asthma.

Keywords: Phosphodiesterase inhibitor; Matrix metalloproteinase; Tissue remodelling; Bronchoalveolar lavage; (Mouse)

1. Introduction

Epithelium basement membrane thickening as a feature of asthmatic airways due to excess in collagen deposition (Roche et al., 1989). Basement membranes are thin extracellular matrix layers underlying epithelium and endothelium, which play a major role in various biological processes such as inflammatory cell migration, cancer, and wound healing. These processes involve matrix metalloproteinases which are able to degrade almost all components of the extracellular matrix (for review, see Corbel et al., 2000). Among them, the gelatinase-A (matrix metalloproteinase-2) and -B (matrix metalloproteinase-9) degrade mainly type IV collagen, the major constituent of basement membranes. Matrix metalloproteinases have been involved in the physiopathology of acute and chronic inflammatory disorders including asthma (Roche et al., 1989; Vignola et al., 1998). Moreover, increased levels of matrix metalloproteinase-9 have been found in bronchoalveolar lavage fluids, bronchial biopsies and alveolar macrophages from asthmatic patients (Mautino et al., 1997; Lemjabbar et al., 1999).

Cyclic nucleotide phosphodiesterase comprise at least seven families of isoenzymes that hydrolyse the 3′,5′-cyclic nucleotides to 5′-nucleotide monophosphates. Among these isoenzymes, type 4 phosphodiesterase appears to be a molecular target for new anti-asthmatic and anti-inflammatory drugs (Barnette, 1999). Indeed, phosphodiesterase 4 enzyme is a major cyclic AMP hydrolysing activity in immune and anti-inflammatory cells and the elevation of intracellular cyclic AMP in these cell types reduces their activity and the release of inflammatory mediators. Moreover, the selective inhibition of phosphodiesterase type 4 isoenzyme by phosphodiesterase 4 inhibitors leads to marked anti-inflammatory activities in vitro and in vivo in several animal models (for review, see Teixeira et al.,

* Corresponding author. Tel.: +33-2-99-33-68-50; fax: +33-2-99-33-62-42.

E-mail address: vincent.lagente@rennes.inserm.fr (V. Lagente).

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1997) including granulocyte recruitment in airways of sensitized and challenged guinea-pigs (Lagente et al., 1994).

In order to assess phosphodiesterase 4 inhibitor as a possible modulator of airway remodelling in asthma, we have investigated the effects of RP 73-401 on matrix metalloproteinase activities in bronchoalveolar lavage fluids of ovalbumin-sensitized and -challenged mice.

2. Materials and methods

2.1. Animals and experimental protocols

Ten week-old male Balb/c mice (CERJ, Le Genest Saint Isle, France) were sensitized by an i.p. injection of a 0.25 ml mixture of ovalbumin (320 μg/ml) adsorbed with aluminium hydroxide [$Al(OH)_3$] (8 mg/ml). One week later, mice received a similar injection. After an additional week, sensitized mice were exposed twice to an ovalbumin aerosol (5 mg/ml for 30 min at first and 10 mg/ml for 30 min 8 h later) in saline solution (0.9% NaCl). Mice exposed to an aerosol of saline solution alone constituted the control group. Aerosols were generated by a Spag-2 nebulisator (ICN Pharmaceuticals, Hyland Ave, California). RP 73-401 [*N*-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide], was synthesized by Dr. J.J. Bourguignon (Faculté de Pharmacie, Illkirch, France), suspended in 1% carboxymethylcellulose (vehicle) and was administered orally 1 h before each ovalbumin challenge at a 0.3–3 mg/kg dose.

2.2. Bronchoalveolar lavage

Twenty-four hours after the last challenge, bronchoalveolar lavage was performed as previously described (Corbel et al., 1999). Bronchoalveolar fluids were centrifuged ($600 \times g$ for 10 min, 4°C) and the supernatants aliquoted and frozen at −80°C until zymography analysis. Cell pellets were resuspended in 0.5 ml RPMI culture medium. Total cell count was performed using an haemocytometer chamber, and cell viability was determined by the trypan blue exclusion method. Then, cytospins were realised at 700 rpm for 10 min (Cytopro 7620 WESCOR), and the cells were stained using May Grünwald-Giemsa. Differential cell counts were calculated on 200 cells using standard morphological criteria.

2.3. Zymography analysis

Zymography analysis was performed as previously described (Corbel et al., 1999). Aliquots of bronchoalveolar lavage fluids were subjected to electrophoresis on acrylamide stacking gel-containing gelatin. The respective activities of matrix metalloproteinase-2 and matrix metalloproteinase-9 were quantified by measuring the intensity of lysis bands of gelatin, using a densitometric analyser (Densylab software, Bioprobe Systems, les Ulis, France). In order to compare all the gels, results were expressed as the percentage of an internal standard band of migration loaded onto each gel.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. Analysis of treatment effects between the various groups was performed with a two-way analysis of variance (ANOVA). Comparison of treatment interactions with control was realised by the Student's *t*-test. For each analysis, *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Total number of cells and cellular composition in bronchoalveolar lavage fluids

Exposure to ovalbumin aerosols led to a significant increase in the number of neutrophils, eosinophils, and

Table 1
Influence of RP 73-401 on cell composition in bronchoalveolar lavage fluids from mice exposed to ovalbumin (OA)

	Dose (mg/kg)	*n*	Total cells	Macrophages	Neutrophils	Eosinophils	Lymphocytes
			Cells × 10^5 ± S.E.M.				
Control	–	11	2.20 ± 0.35	2.10 ± 0.34	0.010 ± 0.001	0 ± 0	0.04 ± 0.02
OA	–	20	2.50 ± 0.29	1.40 ± 0.11[a]	0.50 ± 0.18[b]	0.30 ± 0.08[b]	0.10 ± 0.03[a]
RP73-401 + OA	0.3	10	1.90 ± 0.16	1.50 ± 0.14	0.10 ± 0.02[c]	0.30 ± 0.05	0.10 ± 0.07
	1	10	2.00 ± 0.21	1.80 ± 0.17	0.10 ± 0.04[c]	0.04 ± 0.01[d]	0.03 ± 0.01[c]
	3	6	2.10 ± 0.27	1.90 ± 0.20	0.10 ± 0.09[d]	0.02 ± 0.01[d]	0.05 ± 0.03

RP 73-401 was administrated orally 1 h before each ovalbumin challenge. Bronchoalveolar lavage was performed 24 h after the last challenge.
n = number of mice.
[a] $P < 0.05$;
[b] $P < 0.01$ in comparison with control mice that have been exposed to saline solution alone;
[c] $P < 0.05$;
[d] $P < 0.01$ in comparison with ovalbumin-challenged mice.

lymphocytes in comparison to saline-exposed mice (control group) (Table 1). RP73-401 treatment allowed to a dose-dependent inhibition of the ovalbumin-induced increased eosinophil numbers in bronchoalveolar lavage. This inhibition was significant at 1 and 3 mg/kg RP 73-401 concentrations. Similarly, a significant reduction in neutrophil numbers was also observed following RP 73-401 treatment but at the three tested concentrations (0.3, 1 and 3 mg/kg). RP 7340 treatment also reduced the number of lymphocytes, but only at the dose of 1 mg/kg. In contrast to neutrophils, eosinophils, and lymphocytes, the number of macrophages decreased after ovalbumin exposures in comparison to control mice, and was not modulated by RP 73-401 treatment.

3.2. Zymography analysis

Ovalbumin-challenge induced a marked induction of matrix metalloproteinase-9 activity (both latent and active forms) in bronchoalveolar lavage fluids in comparison to control mice (Fig. 1). When treated with RP 73-401 and whatever the dose tested, this increased matrix metalloproteinase-9 activity was markedly and significantly ($P < 0.05$) inhibited (Fig. 1).

In contrast to that, we have observed for matrix metalloproteinase-9, there was no significant increase in matrix metalloproteinase-2 activity in the bronchoalveolar lavage fluids of mice exposed to antigen, treated or not with RP 73-401.

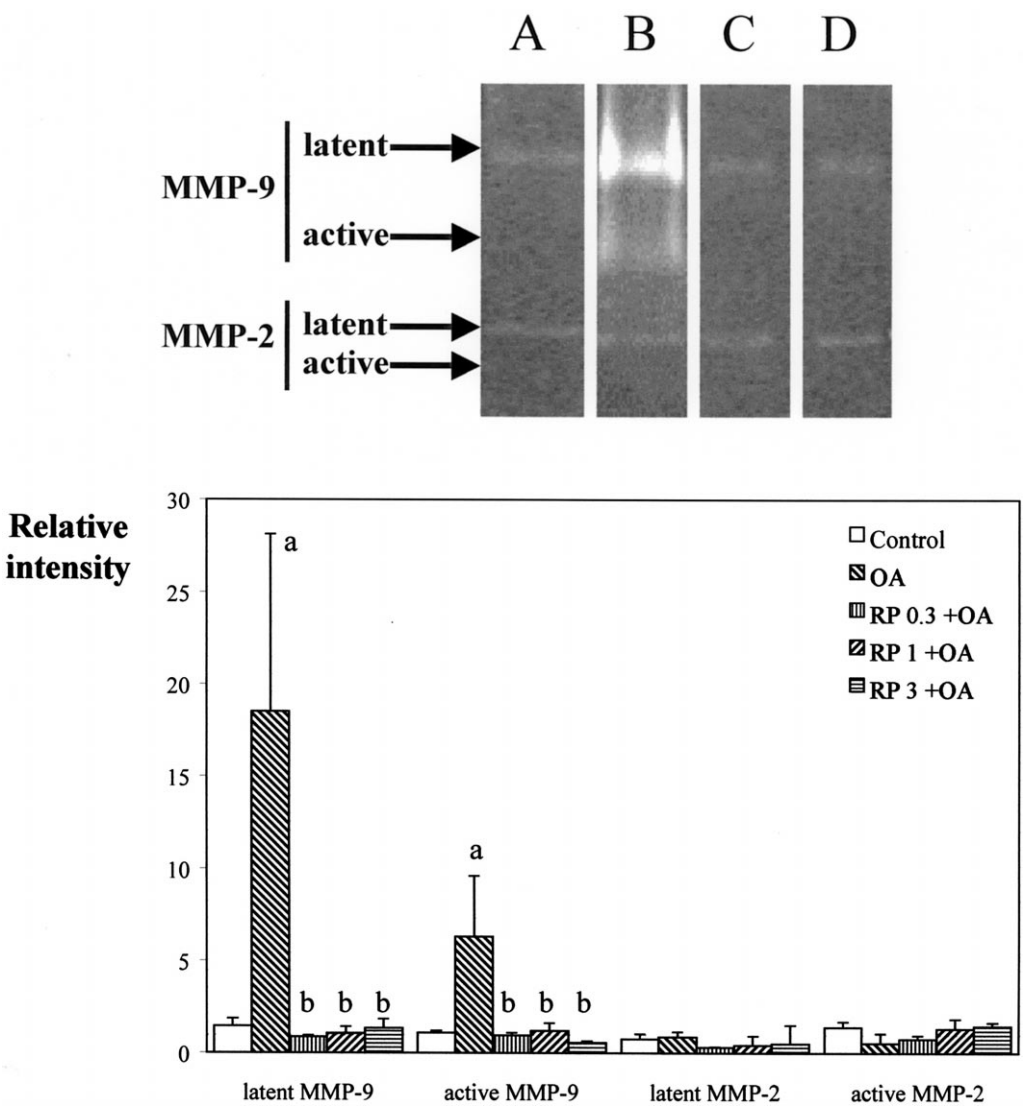

Fig. 1. Influence of RP 73-401 on matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) activities in bronchoalveolar lavage fluids from mice exposed to ovalbumin (OA). Upper panel: Gelatin zymogram performed with bronchoalveolar lavage fluids. Lane A: control mice, Lane B: Bronchoalveolar lavage fluids from ovalbumin-challenged mice; Lane C: Ovalbumin-challenged mice treated with 3 mg/kg RP 73-401; Lane D: Ovalbumin-challenged mice treated with 1 mg/kg RP 73-401. Lower panel: Effects of RP 73-401 (0.3–3 mg/kg) on matrix metalloproteinases in bronchoalveolar lavage fluids recovered from ovalbumin-challenged and control mice. Results were expressed as mean ± S.E.M. of relative percentages of an internal control sample. (a) $P < 0.01$ in comparison with saline-exposed, RP 73-401 non-treated control mice; (b) $P < 0.01$ in comparison with ovalbumin-challenged, RP 73-401 non-treated mice.

4. Discussion

Phosphodiesterase type 4 has now become an important molecular target for the development of novel therapies for inflammatory diseases including asthma (Barnette, 1999; Schmidt et al., 1999). Indeed, several selective phosphodiesterase type 4 inhibitors have been reported to reduce pulmonary inflammation in various experimental models (for review, see Teixeira et al., 1997), including sensitized guinea-pigs (Lagente et al., 1994). Nevertheless, the possible ability of phosphodiesterase 4 inhibitors to prevent or modulate the airway remodelling remains a relatively unexplored area. Airway remodelling is generally the consequence of chronic airway inflammation leading to changes in extra cellular matrix components and involved matrix metalloproteinases.

We presently reported that exposure of Balb/c mice to ovalbumin induced a dramatic recruitment of polymorphonuclear neutrophils and eosinophils in bronchoalveolar lavage fluids which was associated with a marked increase in matrix metalloproteinase-9 activity, whereas matrix metalloproteinase-2 activity was not modulated. Up-regulation of matrix metalloproteinase-9 activity following ovalbumin challenge is consistent with previous studies reporting that (i) matrix metalloproteinase-9 content was increased in bronchoalveolar lavage fluids from untreated asthmatics compared to steroid-treated asthmatics and control subjects (Mautino et al., 1997; Lemjabbar et al., 1999), and (ii) antigen challenge increased matrix metalloproteinase-9 activity in bronchoalveolar lavage fluids from sensitized mice (Kumagai et al., 1999) or asthmatic patients (Warner et al., 1997). Even though several studies suggested that matrix metalloproteinase-9 was mainly secreted by eosinophils in asthma (Ohno et al., 1997), recent reports showed that neutrophils might also constitute a potent source of matrix metalloproteinase-9 (Halliday et al., 1999). In addition, Lemjabbar et al. (1999) suggested that the increase in matrix metalloproteinase-9 levels in bronchoalveolar lavage from status asthmaticus patients may be shared between neutrophils and activated bronchial epithelial cells in response to lung injury.

In our study, matrix metalloproteinase-2 activity was not increased in bronchoalveolar lavage fluids from ovalbumin-exposed mice. Although matrix metalloproteinase-2 and matrix metalloproteinase-9 shared some substrate specificities, these enzymes are well described to be synthesized by different cell types. Matrix metalloproteinase-2 is mainly synthesized by structural cells including fibroblasts, endothelial and epithelial cells, whereas matrix metalloproteinase-9 is produced mainly by inflammatory cells, including eosinophils and neutrophils (Mautino et al., 1997).

Treatment of mice with the selective phosphodiesterase 4 inhibitor, RP 73-401, significantly reduced the influx of neutrophils and eosinophils following ovalbumin challenge. RP 73-401 also markedly reduced the enhanced matrix metalloproteinase-9 activity in the bronchoalveolar lavage fluid of challenged mice. Therefore, it is conceivable that the reduction in the recruitment of inflammatory cells by RP 73-401 is closely associated with the reduction of matrix metalloproteinase-9.

We previously demonstrated that a pre-treatment with betamethasone elicited a reduction in both matrix metalloproteinase-9 activity and the enhanced neutrophil number in bronchoalveolar lavages of lipopolysaccharide-exposed mice. In contrast, in the same model, cyclosporin pre-treatment did not significantly reduce matrix metalloproteinase activities but inhibited neutrophil recruitment, suggesting that both events may be dissociated (Corbel et al., 1999). In the present study, we showed that pre-treatment of mice with 0.3 mg/kg RP 73-401 is accompanied by a significant reduction in neutrophil influx, but not of eosinophil and a decrease in matrix metalloproteinase-9 activity, suggesting that the matrix metalloproteinase-9 activity is linked to the recruitment of neutrophils rather than eosinophils. This is consistent with the results on the increased matrix metalloproteinase-9 activity has been obtained in mice exposed to lipopolysaccharide, where almost all the inflammatory cells recruited in the bronchoalveolar lavage fluid are neutrophils (Corbel et al., 1999).

In conclusion, we demonstrated that RP 73-401, reduces antigen challenge induced-cell recruitment in airways of sensitised mice, but also diminished matrix metalloproteinase-9 activity. These results suggest that selective phosphodiesterase 4 inhibitors might indirectly modulate tissue remodelling associated with inflammatory diseases including asthma.

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References

Barnette, M.S., 1999. Phosphodiesterase 4 (PDE4) inhibitors in asthma and chronic obstructive pulmonary disease (COPD). In: Jucker, E. (Ed.), Progress in Drug Research vol. 53 Birkhäuser Verlag, Basel, Switzerland, pp. 193–229.

Corbel, M., Lagente, V., Théret, N., Germain, N., Clément, B., Boichot, E., 1999. Comparative effects of betamethasone, cyclosporin and nedocromil sodium in acute pulmonary inflammation and metalloproteinase activities in bronchoalveolar lavage fluid from mice exposed to lipopolysaccharide. Pulm. Pharmacol. Ther. 12, 165–171.

Corbel, M., Lagente, V., Boichot, E., 2000. Pulmonary inflammation and tissue remodelling: role of metalloproteinases. Eur. Respir. Rev. 10, 260–263.

Halliday, J.L., Chu, H.W., Langmack, E., Wenzel, S.E., 1999. Matrix metalloproteinase 9 (MMP-9) expression occurs in asthmatic extracellular matrix (ECM) and subbasement membrane (SBM) and is associ-

ated with severity of disease. Am. J. Respir. Crit. Care Med. 159, A325.

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.

Lagente, V., Moodley, I., Perrin, S., Mottin, G., Junien, J.L., 1994. Effects of isozyme selective phosphodiesterase inhibitors on eosinophil infiltration in the guinea-pig lung. Eur. J. Pharmacol. 255, 253–256.

Lemjabbar, H., Gosset, P., Lamblin, C., Tillie, I., Hartmann, D., Wallaert, B., Tonnel, A.B., Lafuma, C., 1999. Contribution of 92 kDa gelatinase/typeIV collagenase in bronchial inflammation during status asthmaticus. Am. J. Respir. Crit. Care Med. 159, 1298–1307.

Mautino, G., Oliver, N., Chanez, P., Bousquet, J., Capony, F., 1997. Increased release of matrix metalloproteinase-9 in bronchoalveolar lavage fluid and by alveolar macrophages of asthmatics. Am. J. Respir. Cell. Mol. Biol. 17, 583–591.

Ohno, I., Ohtani, H., Nitta, Y., Suzuki, J., Hoshi, H., Honma, M., Isoyama, S., Tanno, Y., Tamura, G., Yamauchi, K., Nagura, H., Shirato, K., 1997. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. Am. J. Respir. Cell. Mol. Biol. 16, 212–219.

Roche, W.R., Beasley, R., Williams, J.H., Holgate, S., 1989. Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1, 520–524.

Schmidt, P., Dent, G., Rabe, K.F., 1999. Selective phosphodiesterase inhibitors for the treatment of bronchial asthma and chronic obstructive pulmonary disease. Clin. Exp. Allergy 29 (Supl. 2), 99–109.

Teixeira, M.M., Gristwood, R.W., Cooper, N., Hellewell, P.G., 1997. Phosphodiesterase (PDE) 4 inhibitors: anti-inflammatory drugs of the future? Trends Pharmacol. Sci. 18, 164–171.

Vignola, A.M., Chanez, P., Siena, L., Chiappara, G., Bonsignore, G., Bousquet, J., 1998. Airways remodelling in asthma. Pulm. Pharmacol. Ther. 11, 359–367.

Warner, J.A., Julius, P., Luttmann, W., Kroegel, C., 1997. Matrix metalloproteinases in bronchoalveolar lavage fluid following antigen challenge. Int. Arch. Allergy Immunol. 113, 318–320.