

A Rho kinase inhibitor, Y-27632 inhibits pulmonary eosinophilia, bronchoconstriction and airways hyperresponsiveness in allergic mice

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Abstract

Asthma is a complex inflammatory disorder involving obstruction, constriction, oedema, remodelling and hyperresponsiveness of the airways. These effects are induced by a raft of mediators, many of which exert their actions by stimulating specific G-protein-coupled receptors linked to a signal transduction pathway involving the monomeric GTPase; rho, and a downstream effector; rho kinase. The aim of this study was to determine whether administration of a selective inhibitor of rho kinase, Y-27632, attenuates airway inflammation, bronchoconstriction and hyperresponsiveness in a murine model of acute allergic inflammation. Intranasal administration of Y-27632 caused a dose-dependent inhibition in the number of eosinophils recovered from bronchoalveolar lavage fluid of ovalbumin-sensitised and challenged (allergic) mice. These inhibitory effects of intranasal Y-27632 on pulmonary eosinophilia were accompanied by a significant inhibition of the development of airways hyperresponsiveness in allergic mice. In additional studies, intranasal Y-27632 inhibited methacholine-induced increases in airways resistance in a time-dependent manner. In conclusion, these findings indicate that activation of rho kinase contributes to bronchoconstriction and eosinophil trafficking in murine models of acute allergic airway inflammation and to the development of airway hyperresponsiveness.

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Keywords: Rho kinase; Airway resistance; Bronchoalveolar lavage; Airway hyperresponsiveness; Eosinophils

1. Introduction

Acute and chronic elevations in airway tone are characteristic features of allergic asthma, and contribute significantly to the obstructive nature of this disease. Airway tone may be elevated by neurotransmitters released from nerves (acetylcholine, sensory neuropeptides) or by mediators released from resident or infiltrating inflammatory cells (histamine, cysteinyl leukotrienes, endothelin-1). Each of these bronchoconstrictor agents exerts its actions via stimulation of specific G protein-coupled receptors located on the surface of airway smooth muscle cells. Receptor stimulation is associated with a rise in the concentration of

cytosolic free Ca^{2+} and the activation of rhoA, a small monomeric GTPase. These two events contribute to airway smooth muscle contraction through distinct mechanisms. Increased cytosolic free Ca^{2+} activates myosin light chain kinase, which phosphorylates myosin light chain and induces airway smooth muscle contraction [1]. In contrast, rhoA induces contraction by stimulating rho kinase, which phosphorylates and thereby inhibits myosin light chain phosphatase [2]. Accordingly, numerous studies have demonstrated that inhibitors of rho kinase are effective relaxants of airway smooth muscle in vitro and are effective bronchodilators in vivo. For example, an inhibitor of rho kinase, Y-27632 [3], relaxed human isolated bronchial preparations [4,5] and inhibited increases in airways resistance induced by acetylcholine in anaesthetised guinea-pigs [6]. Moreover, the translocation of rhoA from the cytosol to the plasma membrane, an indicator of rhoA activation, was increased in bronchial smooth muscle preparations from allergic rats [7] and Y-27632 inhibited

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methacholine-induced increases in airways resistance in an acute murine model of allergic inflammation [8]. Together, these latter findings suggest that the rho/rho kinase signalling pathway may play an important role in the elevated airway tone associated with allergen-induced respiratory disease, and that inhibition of this pathway might be a novel bronchodilator approach for allergic asthma.

In addition to airway smooth muscle contraction, significant contributions to the airway obstruction of asthma are made by factors associated with airway inflammation and remodelling [9]. The asthmatic inflammatory response is complex and not fully understood, but numerous studies have highlighted the obligatory role of CD4⁺ Th2 lymphocytes and the importance of eosinophils in the aetiology of asthma [10]. The recruitment of eosinophils from the circulation to sites of allergic inflammation is a multistep process and appears to be governed by the generation of eosinophil-specific chemoattractants, such as eotaxin, and the activation of cell-surface adhesion molecules preferentially expressed on eosinophils [11]. Recent *in vitro* studies have provided compelling evidence that activation of rhoA and rho kinase is essential for eotaxin-induced eosinophil chemotaxis [12] and for the movement of eosinophils through monolayers of cultured endothelial cells [13]. Rho kinase activity appears to be restricted to the rear of the eosinophil to generate the retraction force required for cell migration. Despite this exciting link between rhoA/rho kinase and eosinophil chemotaxis, no work has been reported that evaluates the impact of the rho kinase inhibitor Y-27632 on eosinophil trafficking following allergen challenge, or the downstream sequelae of eosinophil activation such as airway hyperresponsiveness.

Thus, the principal aim of the current study was to evaluate the influence of Y-27632 on the development of pulmonary eosinophilia, airways inflammation and airway hyperresponsiveness in ovalbumin-sensitised and challenged mice.

2. Methods

2.1. Animals

Specified pathogen-free, female BALB/c mice, 6 weeks of age at the commencement of the study (Animal Resource Centre, Murdoch, Australia), were used throughout these studies. All experiments had approval from the University of Western Australia Animal Ethics Committee, and adhered to guidelines established by the National Health and Medical Research Council of Australia.

2.2. Antigen exposure and drug treatment of mice

Groups of mice were sensitised to ovalbumin (Sigma, St Louis, MO) by intraperitoneal injection (20 µg ovalbumin

plus 2.25 mg alhydrogel, i.p.) on days 0 and 14, and by intranasal instillation (20 µg ovalbumin in 20 µL) on day 14. For intranasal instillations, solutions were applied to the noses of anaesthetised mice (methoxyflurane). Sham-sensitised mice, which received ovalbumin-free solutions on day 0 and 14, acted as controls. Two different ovalbumin challenge protocols were used. Groups of ovalbumin-sensitised and sham-sensitised mice were anaesthetised (methoxyflurane) and challenged with a single dose of ovalbumin administered intranasally (125 µg in 20 µL) on day 25 (single intranasal challenge model, Fig. 1A) or on days 25, 26 and 27 (triple intranasal challenge model, Fig. 4A). Y-27632 (Tocris Cookson, Avonmouth, UK) or sterile saline was administered intranasally 1 h prior to each of the ovalbumin challenges (see Figs. 1A and 4A for protocols).

2.3. Bronchoalveolar lavage (BAL)

At selected times post-challenge, mice were sacrificed (pentobarbitone, 200 mg/kg i.p.) and their lungs lavaged *in situ* via a tracheal cannula with 5 × 0.5 mL aliquots of PBS. BAL fluid was centrifuged (1500 rpm for 10 min), the cell pellet resuspended in PBS plus 1% BSA, and total cell number and viability were calculated using a haemocytometer and trypan blue exclusion, respectively. Scoring a minimum of 400 cells/preparation, differential cell counts of macrophages/monocytes, lymphocytes, neutrophils and eosinophils were performed on cytopspin preparations stained with DiffQuik (Lab Aids, Narrabeen, Australia).

2.4. Histology

Mice were killed with an overdose of pentobarbitone (200 mg/kg) and lungs were fixed with Bouin's fixative via pulmonary perfusion and a 4 h post-fixation period. The tissue was subsequently washed, dehydrated and embedded in paraplast. Tissue sections of 5 µm thickness were stained with Mayers haematoxylin and eosin for assessment of infiltrating cells and general lung architecture. Mucopolysaccharide-producing cells were identified using Periodic Acid Schiff (PAS) staining (Schiff reagent: Australian Biostain, Vic., Australia).

2.5. Intravascular eosinophils

Haematoxylin and eosin stained 5 µm paraffin sections from the mid zone of the left lobe were examined by bright field microscopy and images captured with an Olympus DP11 digital camera system (Olympus Optical Co., Ltd Tokyo, Japan). The diameter of blood vessels was measured via Image-Pro^R Plus 'The Proven SolutionTM' software calibrated with a micron reference measurement slide. Enumeration of inflammatory leukocytes (eosinophils) in vessels of 15–50 µm was performed using an Olympus X40 UPlan FI objective and counted blind (by TSM). The data were used to express the mean number of cells per vessel.

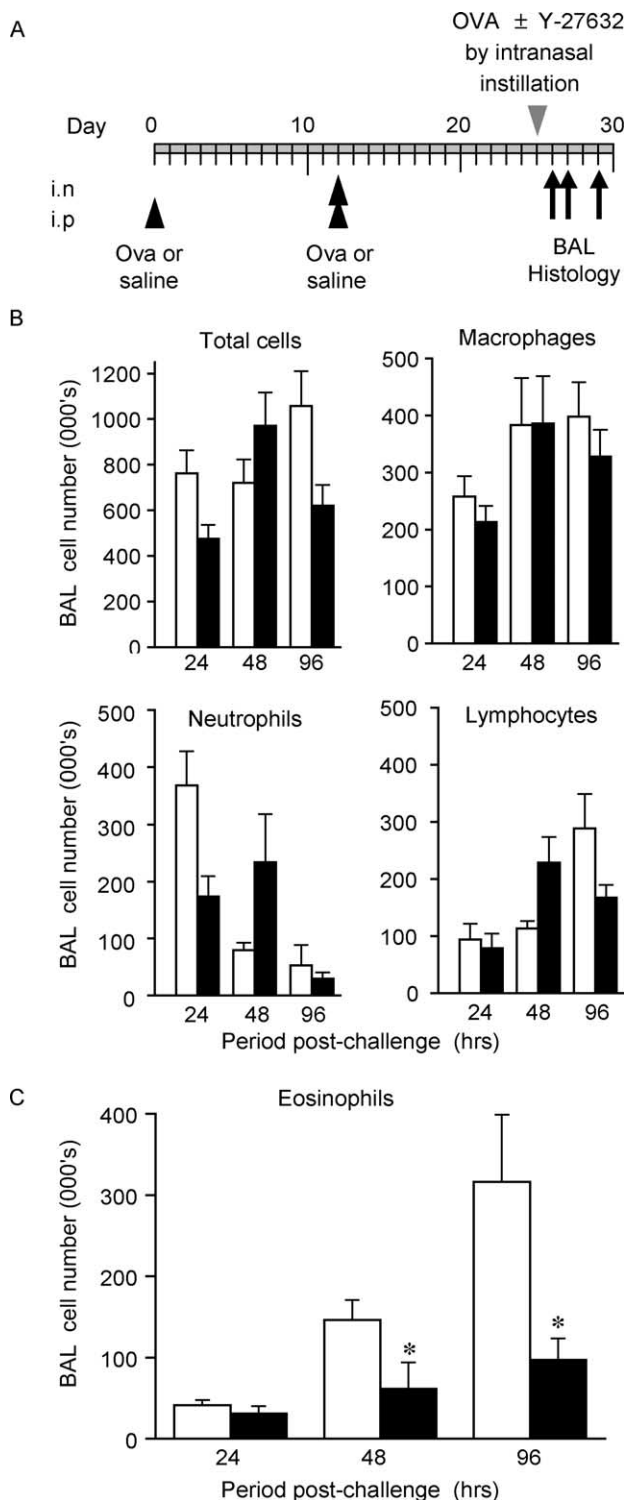


Fig. 1. (A) A single intranasal challenge model was used to investigate the influence of intranasally instilled Y-27632 on the cell profile of BAL fluid obtained from allergic and non-allergic mice. At 24, 48 and 96 h post-challenge, BAL fluid was obtained from allergic mice and the numbers of total cells, macrophages, neutrophils, lymphocytes (B) and eosinophils (C) was determined. Shown are mean $\pm$ s.e.m. responses obtained from allergic mice treated with either intranasal saline (white bars) or 10 mg/kg Y-27632 (black bars). $n=7$ mice per group (* $P<0.05$ cf time-matched, saline-treated mice).

2.6. In vivo lung function recordings

On day 29, 2 days following the last intranasal challenge with ovalbumin, selected triple intranasal challenge mice were anaesthetised (ketamine 100 mg/kg, xylazine 10 mg/kg, i.p.) and cannulae placed in the trachea (to measure airflow), jugular vein (for drug administration) and oesophagus (for transpulmonary pressure). Spontaneous breathing was stopped by pancuronium bromide (0.4 mg/kg, i.v., AstraZeneca, North Ryde, Australia) and thereafter mice were ventilated using a SAR-830 ventilator (CWE, Inc., Ardmore, PA, USA) at 150 breaths per minute with a tidal volume of 3.5 ml/kg. Breath-to-breath measurement of airway resistance (R_L) was recorded (PMS800, Mumed, London, UK) according to the principles of Amdur and Mead [14]. To investigate the development of airways hyperresponsiveness, increasing bolus doses of a bronchoconstrictor stimulus methacholine (62.5, 125, 250, and 500 μ g/kg, i.v.; Sigma, St Louis, MO) were administered to allergic mice at 5 min intervals, and the dose–response relationship compared to that obtained in non-allergic mice.

In additional experiments, the duration of action of intranasal Y-27632 was determined by measuring airways responsiveness to methacholine (62.5–500 μ g/kg, i.v.) in groups of mice that had received 10 mg/kg Y-27632 or saline intranasally at 2, 4, 8 or 24 h earlier.

2.7. Statistical analyses

Unless otherwise stated, data are expressed as mean $\pm$ s.e.m. Groups of data were compared by analysis of variance (SigmaStat software), and where appropriate a modified t -statistic was used to test for differences between means of specific pairs of groups [15].

3. Results

3.1. Effect of Y-27632 on BAL fluid cell profile following single intranasal challenge in allergic mice

Using the protocol shown in Fig. 1A, we investigated the effect of intranasal Y-27632 on the cellular composition of BAL fluid obtained from allergic mice at selected periods following single intranasal challenge with ovalbumin. Ovalbumin challenge induced a rapid, but transient, increase in the number of neutrophils in BAL fluid recovered from ovalbumin-sensitised mice (Fig. 1B). As expected, the early neutrophilia was followed by a later peak in the numbers of BAL lymphocytes (Fig. 1B) and eosinophils (Fig. 1C). Intranasal administration of 10 mg/kg Y-27632 to allergic mice was associated with significant changes in both total and differential cell counts. The most prominent feature was a marked reduction in the number of BAL eosinophils, such that at 48 and 96 h post-challenge,

Y-27632-treated allergic mice had 58 and 70% fewer BAL eosinophils than vehicle-treated allergic mice (Fig. 1C). In addition, Y-27632 pretreatment was associated with a small delay in the peak increase in BAL neutrophils from 24 to 48 h (Fig. 1B).

3.2. Effect of different doses of Y-27632 on numbers of BAL eosinophils following single intranasal challenge in allergic mice

In the single intranasal challenge model, 3 mg/kg Y-27632 did not significantly reduce the numbers of BAL eosinophils recovered from allergic mice 96 h post-challenge (Fig. 2). In contrast, both the 10 and 30 mg/kg doses of Y-27632 were associated with a significant reduction in the numbers of BAL eosinophils (Fig. 2), although the greatest reduction was produced by 10 mg/kg Y-27632.

3.3. Effect of Y-27632 on intravascular eosinophils and lung pathology

Further studies were performed to determine whether the inhibitory effect of Y-27632 on the recovery of BAL eosinophils from allergic mice was due to impaired movement of eosinophils across the vasculature endothelium, as indicated by recent *in vitro* findings [12,13]. Visual inspection of lung sections revealed significant numbers of eosinophils on the luminal side of the vascular wall of allergic mice, but not of non-allergic mice (Fig. 3A and a–c). A quantitative evaluation established that there

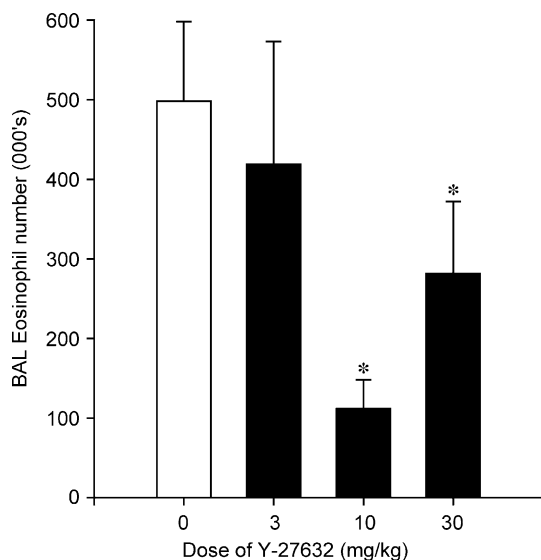


Fig. 2. Numbers of eosinophils recovered from BAL fluid of allergic mice following pretreatment with 0 (vehicle, white bar) or 3, 10 or 30 mg/kg Y-27632 (black bars). BAL was performed 96 h after mice had received a single intranasal ovalbumin challenge. Shown are mean $\pm$ s.e.m. responses obtained using 5–6 mice per group (* $P < 0.05$ cf vehicle).

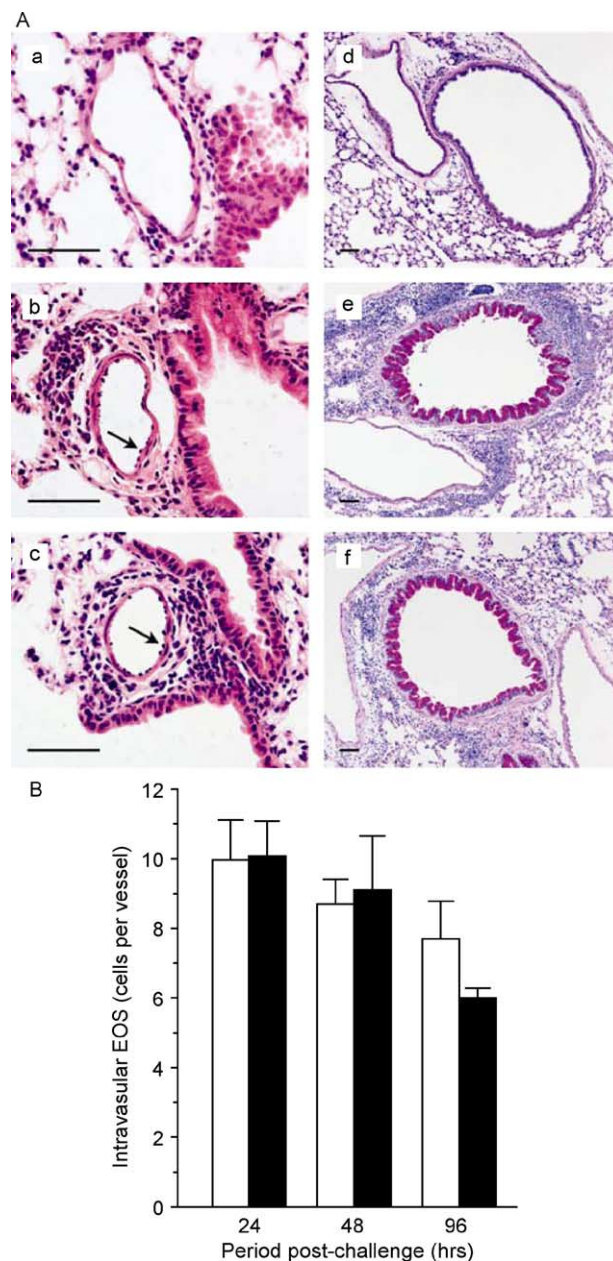


Fig. 3. (A) Lightfield photomicrographs of lung sections obtained from saline-treated non-allergic mice (a and d), saline-treated allergic mice (b and e), and Y-27632-treated allergic mice (c and f). Tissue sections have been stained with either haematoxylin and eosin (a–c) or Periodic-Acid Schiff's reagent (d–f). In (b) and (c), the vascular endothelium (white arrow) and selected intraluminal eosinophils (black arrow) have been highlighted. Bar = 100 μ m. (B) Number of intravascular eosinophils in the lungs of allergic mice treated with either saline or Y-27632 (10 mg/kg), determined 24, 48 and 96 h after mice had received a single intranasal ovalbumin challenge (protocol, Fig. 1A). Between 7 and 17 blood vessels of between 15 and 50 μ m in diameter were examined in each mouse. Shown are mean $\pm$ s.e.m. responses obtained from four mice per group.

were more intraluminal eosinophils present at 24 h post-challenge than at 96 h post-challenge (Fig. 3B). However, there were similar numbers of intraluminal eosinophils in saline- and Y-27632-treated allergic mice (Fig. 3B). Levels

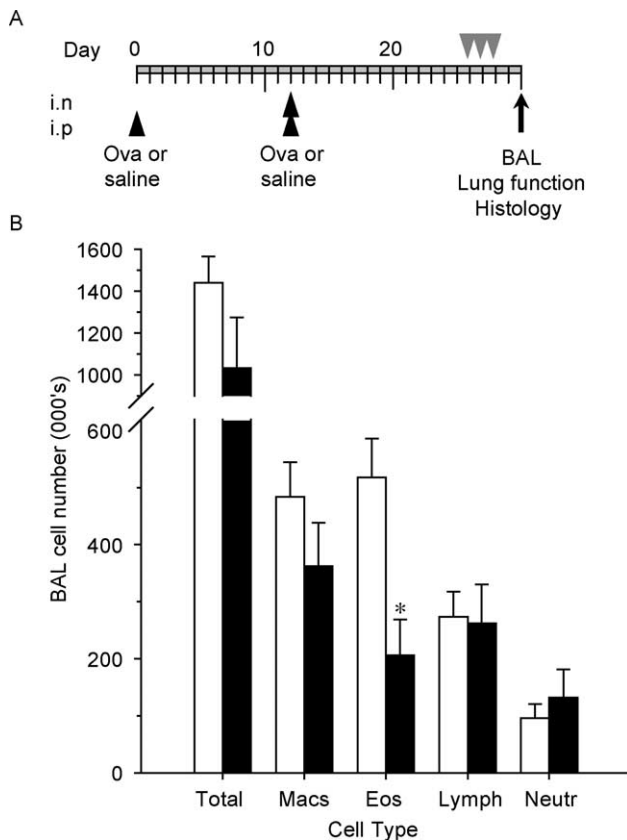


Fig. 4. (A) A triple intranasal challenge model was used to investigate the influence of intranasally instilled Y-27632 on airway inflammation and hyperresponsiveness in allergic and non-allergic mice. (B) Total and differential cell counts obtained from the BAL fluid of allergic mice treated with either intranasal saline (white bars) or Y-27632 (10 mg/kg, black bars), determined 48 h after the final of three intranasal ovalbumin challenges. Shown are mean $\pm$ s.e.m. responses obtained from seven mice per group (* $P < 0.05$ cf saline-treated mice).

of PAS staining over bronchial epithelium were similarly elevated in Y-27632- and vehicle-treated allergic mice, compared to non-allergic mice (Fig. 3A and d–f).

3.4. Effect of Y-27632 on BAL fluid cell profile following triple intranasal challenge in allergic mice

Additional experiments were performed to investigate the effects of Y-27632 in a more complex model of allergic inflammation, in which ovalbumin-sensitised mice were repeatedly challenged on 3 consecutive days, and BAL fluid recovered 48 h after the final challenge (see protocol, Fig. 4A). This protocol induced a robust pulmonary eosinophilia, such that approximately 35% of BAL cells recovered from allergic mice were eosinophils (Fig. 4B). Of particular interest, BAL fluid from allergic mice pretreated with intranasal Y-27632 (10 mg/kg) had 60% fewer eosinophils ($206,000 \pm 62,000$, $n = 7$) than that from saline-treated allergic mice ($518,000 \pm 68,000$, $n = 7$, $P < 0.05$). Similar numbers of intraluminal eosinophils were counted in saline-treated (10.9 ± 2.2 eosinophils/vessel,

$n = 4$ mice) and Y-27632-treated (10.5 ± 1.7 eosinophils/vessel, $n = 4$ mice) mice. In this triple challenge model, intranasal Y-27632 had no significant effect on the numbers of macrophages, lymphocytes or neutrophils recovered by bronchoalveolar lavage of allergic mice (Fig. 4B).

3.5. Effect of intranasal Y-27632 on airways hyperresponsiveness following triple intranasal challenge in allergic mice

The bronchoconstrictor methacholine caused larger increases in airway resistance in allergic mice than in non-allergic mice, that is, allergic mice were hyperresponsive (Fig. 5). For example, 250 $\mu\text{g/kg}$ methacholine increased airway resistance by 885 ± 35 cm H₂O/L/s in allergic mice ($n = 6$) and 423 ± 36 cm H₂O/L/s in non-allergic mice ($n = 9$, $P < 0.05$). However, pretreatment of allergic mice with intranasal Y-27632 (10 mg/kg) 1 h before each of the three ovalbumin challenges caused a significant reduction in responsiveness to methacholine (250 $\mu\text{g/kg}$ methacholine increased resistance by 614 ± 34 cm H₂O/L/s, $n = 7$, $P < 0.05$ cf saline-treated allergic mice; Fig. 5). In contrast, intranasal instillation of Y-27632 to non-allergic mice had no significant effect on responsiveness to methacholine (e.g. 250 $\mu\text{g/kg}$ methacholine, 476 ± 68 cm H₂O/L/s, $n = 6$; Fig. 5). These latter findings suggest that the inhibitory effects produced by Y-27632 in allergic mice were not due to direct suppression of methacholine-induced increases in airways resistance, but rather involved separate pathways linked to the development of airway hyperresponsiveness. To confirm this, we investigated the duration of action of

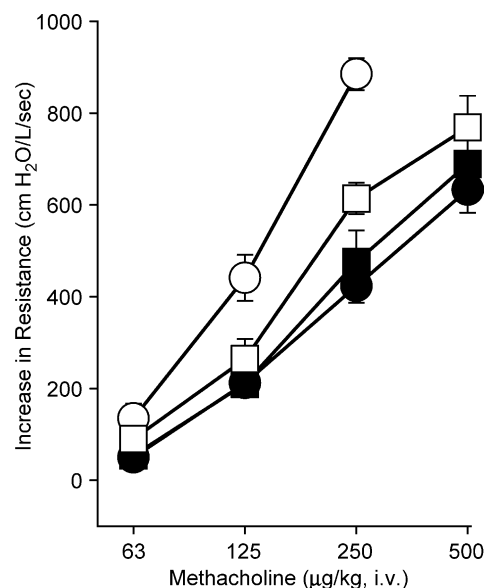


Fig. 5. Dose–response relationship for methacholine-induced increases in airways resistance obtained in allergic (open symbols) and non-allergic (filled symbols) mice, treated with either intranasal Y-27632 (squares) or saline (circles), according to the protocol outlined in Fig. 4A. Shown are mean $\pm$ s.e.m. responses obtained from six to nine mice per group.

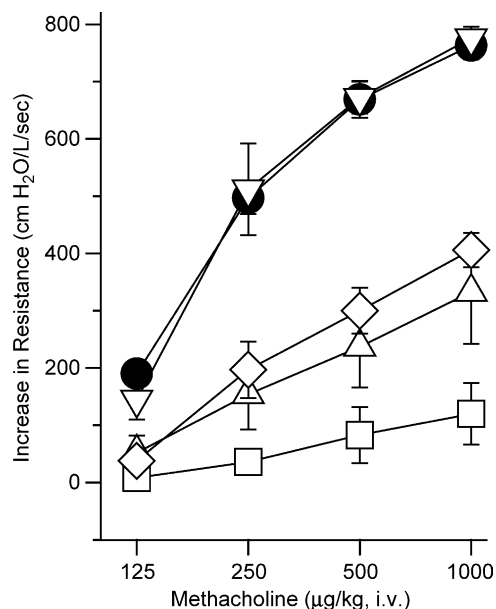


Fig. 6. Methacholine dose-response curves determined in non-allergic mice, 2 (□, $n=6$), 4 (△, $n=6$), 8 (◇, $n=5$) and 24 (▽, $n=4$) h after an intranasal instillation of Y-27632 (10 mg/kg), or saline (•, $n=20$).

Y-27632 as a functional antagonist of methacholine-induced increases in airways resistance. Intranasal Y-27632 markedly inhibited methacholine-induced increases in airways resistance for 2, 4 and 8 h post-instillation, but had no observable effect at 24 h post-instillation (Fig. 6). Thus, it is unlikely that the inhibitory effect of Y-27632 observed in allergic mice at 48 h post-challenge (Fig. 5) was due to any residual bronchodilator action of Y-27632.

4. Discussion

In this study, intranasal administration of a selective inhibitor of rho kinase, Y-27632, to allergic mice was associated with several potentially beneficial effects, including a reduction in the numbers of BAL eosinophils, inhibition of the development of airway hyperresponsiveness, and functional antagonism of spasmogen-induced bronchoconstriction.

Pretreatment with Y-27632 caused a significant reduction in the number of eosinophils that were recovered from the BAL fluid of ovalbumin-sensitised mice following either a single or triple intranasal ovalbumin challenge. In vitro studies have demonstrated that Y-27632 retards the movement of eosinophils through monolayers of cultured endothelial cells, by inhibiting the detachment of the rear end of the cell [13]. If this mechanism was operational in vivo, we anticipated that eosinophils would accumulate within the blood vessels of Y-27632-treated allergic mice, as has previously been observed in mice that are deficient in

CCR3, a receptor that recognises the chemokine eotaxin [16]. However, histological examination of lung sections and quantitative assessment of the numbers of intraluminal eosinophils revealed no enhanced accumulation of intraluminal eosinophils in intrapulmonary blood vessels of Y-27632-treated allergic mice. These findings indicate that Y-27632 did not inhibit pulmonary eosinophilia in the current study by directly impeding the movement of eosinophils through the vascular endothelium. Thus, the precise mechanism through which Y-27632 inhibits pulmonary eosinophilia is not clear. One possible explanation for our findings is that Y-27632 blunts the recruitment of eosinophils by inhibiting upstream signalling events that are involved in recruiting eosinophils into the airways during an allergic inflammatory response. Consistent with this, several studies have demonstrated that Y-27632 reduces the in vitro function of dendritic cells [17] and Th2 lymphocytes [18], which are known to play fundamental roles in orchestrating the recruitment of eosinophils into the airways following allergen challenge. Future studies will also examine the effects of Y-27632 on allergen-induced cytokine, chemokine and adhesion molecule expression.

Intranasal administration of Y-27632 to ovalbumin-sensitised and challenged mice caused a dose-dependent inhibition of pulmonary eosinophilia. However, the dose of Y-27632 that inhibited the influx of eosinophils most effectively (10 mg/kg) did not completely abolish the influx of eosinophils. Similarly, Shimizu and workers [19] reported that bleomycin-induced lung fibrosis was only partially inhibited by intraperitoneal administration of Y-27632, and raised the possibility that continuous delivery of Y-27632 using an osmotic minipump may have provided a more complete inhibition. In additional studies, we observed that administration of Y-27632 via osmotic minipump at the same daily dose used for intranasal dosing (10 mg/kg) did not produce any significant inhibition of pulmonary eosinophilia (data not shown). A possible explanation for these apparently disparate findings is that administration of intranasal Y-27632 an hour before ovalbumin challenge produces transient, but high peak concentrations of Y-27632 locally in the lung, which then inhibit processes involved in subsequent pulmonary eosinophilia. In contrast, administration of Y-27632 via osmotic minipump is likely to produce a level of Y-27632 in the airways that is relatively constant, but not high enough to effectively inhibit pulmonary eosinophilia. Although the administration of higher doses of Y-27632 via osmotic minipump (30 mg/kg/day) have been reported to inhibit the development of hypoxic pulmonary hypertension, a 60 mg/kg/day dose caused early death in mice [20]. Together, these findings indicate that additional Y-27632-insensitive mechanisms also contribute to eosinophil trafficking in allergic airways.

In addition to having fewer BAL eosinophils, allergic mice pretreated with intranasal Y-27632 1 h prior to each allergen challenge were also significantly less responsive

to the bronchoconstrictor methacholine. It was important to distinguish whether these inhibitory effects of Y-27632 on methacholine-induced increases in airway resistance were the result of a direct functional antagonism of methacholine-induced bronchoconstriction (i.e. a bronchodilator effect) or rather, reflect a hitherto unrecognised ability of Y-27632 to inhibit the development of airways hyperresponsiveness. It is well established that inhibitors of rho kinase are effective relaxants of airway smooth muscle *in vitro* and are effective bronchodilators *in vivo* [4–6,8], and in the current study, intranasal administration of Y-27632 markedly inhibited methacholine-induced increases in airways resistance. However, as expected, the bronchodilator activity of Y-27632 waned with time and 24 h post-instillation no bronchodilator effect was evident. Similarly, Hashimoto and coworkers [8] reported that the inhibitory effects of orally administered Y-27632 on methacholine- (and serotonin-) induced increases in lung resistance did not last beyond 4–8 h in ovalbumin-sensitised and challenged BALB/c mice. Thus, it is improbable that any residual bronchodilatory effects of intranasal Y-27632 would have existed at 48 h post-instillation, the time-point when measurements of airway responsiveness to methacholine in allergic mice were performed. Further direct evidence for this postulate was obtained from the findings that airways responsiveness to methacholine was similar in groups of non-allergic (not sensitised to ovalbumin) mice that received intranasal Y-27632 or vehicle 1 h prior to each of three ovalbumin challenges. Together, these findings demonstrate for the first time that pretreatment of allergic mice with intranasal Y-27632 attenuates the development of airways hyperresponsiveness.

The findings of the current study that pretreatment of allergic mice with intranasal Y-27632 significantly inhibited both pulmonary eosinophilia and the development of airways hyperresponsiveness are consistent with a number of studies reporting that pulmonary eosinophilia is positively correlated with a range of histopathological and functional changes in the allergic airway, including airway hyperresponsiveness. Inhibiting eosinophil trafficking could conceivably impair the development of airway hyperresponsiveness in allergic mice by suppressing several important changes in the airway wall that have been proposed to contribute to airway hyperresponsiveness, including thickening of the airway wall, damage to and loss of barrier function of the epithelium, and the release of mediators that promote airway smooth muscle contraction [9]. The current study did not seek to determine a causal link between pulmonary eosinophilia and airway hyperresponsiveness, although it is relevant to note that two recent studies have demonstrated such a link in ovalbumin-sensitised and challenged mice [21,22].

Y-27632 is a potent and specific inhibitor of rho kinase [3] and has been widely used to evaluate the functional importance of rho kinase *in vitro* and *in vivo*. In the current

study, Y-27632 was administered intranasally and found to inhibit airway eosinophilia, bronchoconstriction and airways hyperresponsiveness. Since Y-27632 seldom inhibits other kinases, it is likely that these effects were caused by inhibition of rho kinase. Nevertheless, as is the case for any *in vivo* study, we cannot be unequivocally sure that the intranasal dosing regimen used in this study produced cellular concentrations of Y-27632 that specifically inhibited rho kinase, and thus we cannot rule out the possibility that actions of Y-27632 at other sites may have contributed to the observed effects.

In conclusion, the current study has established that inhibition of rho kinase suppresses bronchoconstriction, eosinophil trafficking and the development of airway hyperresponsiveness in murine models of acute allergic airway inflammation.

Acknowledgements

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