Mast cell mediators cause early allergic bronchoconstriction in guinea-pigs in vivo: a model of relevance to asthma

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Abstract
One feature of allergic asthma, the EAR (early allergic reaction), is not present in the commonly used mouse models. We therefore investigated the mediators involved in EAR in a guinea-pig in vivo model of allergic airway inflammation. Animals were sensitized using a single OVA (ovalbumin)/alum injection and challenged with aerosolized OVA on day 14. On day 15, airway resistance was assessed after challenge with OVA or MCh (methacholine) using the forced oscillation technique, and lung tissue was prepared for histology. The contribution of mast cell mediators was investigated using inhibitors of the main mast cell mediators [histamine (pyrilamine) and CysLTs (cysteinyl-leukotrienes) (montelukast) and prostanoids (indomethacin)]. OVA-sensitized and challenged animals demonstrated AHR (airway hyper-responsiveness) to MCh, and lung tissue eosinophilic inflammation. Antigen challenge induced a strong EAR in the sensitized animals. Treatment with a single compound, or indomethacin together with pyrilamine or montelukast, did not reduce the antigen-induced airway resistance. In contrast, dual treatment with pyrilamine together with montelukast, or triple inhibitor treatment, attenuated approximately 70 % of the EAR. We conclude that, as in humans, the guinea-pig allergic inflammation model exhibits both EAR and AHR, supporting its suitability for in vivo identification of mast cell mediators that contribute to the development of asthma. Moreover, the known mast cell mediators histamine and leukotrienes were major contributors of the EAR. The data also lend further support to the concept that combination therapy with selective inhibitors of key mediators could improve asthma management.

Key words: airway hyper-responsiveness (AHR), animal model, asthma, combination treatment, early allergic reaction, guinea-pig, mast cell

INTRODUCTION
As repeated mast cell activation is one fundamental step in the induction of chronic airway inflammation in asthmatic patients [1,2], there is a great need to have an animal model where mast cell activation occurs and where the mediators of the mast cell-dependent response are the same as in humans. Such a model would be predictive for investigating mechanisms with relevance to bronchoconstriction in subjects with asthma. Today mice are the most commonly used species for experimental asthma research. Although specific mechanisms have been discovered in mice models of asthma, these have not successfully translated into new asthma treatments.

As pointed out in a recent review, one reason for this failure of the mice models may be that the mast cell distribution and reactions in mice, in several respects, are different from those in humans [3]. The mast cells in mice are few and mainly located in the trachea and larger bronchi with almost none in the smaller airways and parenchyma [4,5]. Moreover, although the mast cells secrete histamine, CysLTs (cysteinyl-leukotrienes) and prostanoids, none of these mediators causes smooth muscle contractions in mice. Instead, only a small indirect smooth muscle contraction to allergen-challenge can be obtained in vitro in mice through the release of 5-HT (5-hydroxytryptamine) that acts through neuronal stimulation and epithelial cell release of acetylcholine [6,7]. The combination of the low number of mast cells, their proximal...
distribution and the weak contractile effect of mast cell mediators in the reactions to allergens also makes it difficult to achieve any increase in resistance to direct allergen exposure in vivo [8].

It is indicated that the EAR (early allergic reaction) in both humans and guinea-pigs involves the release and action of histamine and CysLTs [9–12]. With respect to PGs (prostaglandins), there are data supporting that PGD$_2$ or TXA$_2$ (thromboxane A$_2$) acting on TP receptors contributes in humans [11] and guinea-pigs in vitro [13], but the relative role of all three mediators has not been investigated in vivo in humans nor in the guinea-pig. Investigations to inhibit the mediator of this response in the guinea-pig have previously been done in ex vivo models of antigen-induced airway contractions, such as parenchymal strips [14], precision cut lung slices and isolated perfused and ventilated lung. However, these experiments were performed in preparations where tissues were challenged in vitro, in the absence of airway inflammation. In this study, we wanted to extend the earlier experiments to an in vivo model where AHR (airway hyper-responsiveness) and tissue inflammation had been induced by prior in vivo challenge with antigen.

To define the role of the main mast cell mediators, histamine, Cys-LTs and prostanoids, that cause bronchoconstriction during allergic airway inflammation, we set up a protocol with both in vivo sensitization and challenge with the antigen OVA (ovalbumin) in guinea-pigs. The airway reaction to aerosolized OVA was measured by the forced-oscillation technique. A systematic pharmacological analysis was undertaken to delineate the role of the main mast cell mediators that cause bronchoconstriction during EAR. This involved a step-wise single and combined inhibition of the histamine H$_1$ receptor, the CysLT$_1$ receptor and COX (cyclo-oxygenase) enzyme. The allergic airway inflammation was confirmed by measurement of AHR induced by MCh (methacholine) and histological analysis of airway inflammation. In addition the distribution of mast cells was investigated.

**MATERIALS AND METHODS**

**Guinea-pig model of allergic sensitization and airway inflammation**

All studies were conducted in accordance with local Janssen Institutional Animal Care and Use Committee (IACUC) guidelines under an approved animal use protocol. Male Dunkin–Hartley guinea-pigs were obtained from Charles Rivers Laboratories, and housed in isolated ventilated cages under specific pathogen-free conditions with a 12 h/12 h light–dark cycle. Animals were given free access to standardized food, hay and water. At the age of 5 weeks and approximately 350 g, sensitization towards OVA (grade V; Sigma–Aldrich) was induced by an intraperitoneal injection of 100 $\mu$g of OVA, together with 100 mg of aluminium hydroxide (Sigma–Aldrich), in a total volume of 1 ml (Figure 1). After 14 days, animals were placed in a translucent plexiglass chamber and exposed to an aerosol challenge of 0.1 % OVA solution in saline using a pressurized-air-driven nebulizer (LC Plus; PARI) for a period of 1 h.

![Figure 1 Allergic OVA guinea-pig model](image)

**Pharmacological intervention of allergen-induced EAR**

The study included treatments with the selective CysLT$_1$ receptor antagonist montelukast (10 mg/kg of body weight), the selective histamine H$_1$ receptor antagonist pyrilamine (10 mg/kg of body weight) and the non-selective COX inhibitor indomethacin (2 mg/kg of body weight), either given as a single compound, combinations of two compounds, or a triple combination intraperitoneally. Treatments were given 30 min before the in vivo aerosol challenge on day 14 as well 6 h (hrs) thereafter and 30 min before the terminal challenge (large arrows).

**Lung function measurements**

Guinea-pigs were anaesthetized with xylazine (15 mg/kg of body weight), followed by Euthasol$^\text{®}$ (45 mg/kg of body weight) intraperitoneally, 24 h after OVA aerosol challenge. When surgical depth of anaesthesia was confirmed, the animals were tracheostomized and attached to a computer-controlled small animal ventilator (FlexiVent; SCIREQ) to measure airway resistance by the forced-oscillation technique using a single-compartment model. The animals were ventilated at a respiration rate of 60 strokes/min, a tidal volume of 9 ml/kg of body weight and a positive end expiratory pressure of 3 cm H$_2$O. When connected to the respirator, saline and one concentration of OVA (10 mg/ml in saline) was administered by an in-line ultrasonic nebulizer (Aeroneb; test experiments revealed no further increase in airway resistance with doses higher than 10 mg/ml of OVA). Aerosol challenges were administered for 10 s, and changes in airway resistance were continuously monitored for 26 min. In a separate set of experiments to evaluate AHR, a dose–response curve to MCh was obtained (0.1, 0.3 and 1 mg/ml).

**Histological investigations**

For histological evaluation of lung tissue, naïve as well as sensitized and challenged guinea-pigs were killed with an
overdose of Euthasol® (150 mg/kg of body weight). The trachea was exposed and cannulated with a 14-gauge luer stub. The lungs were inflated in situ to a pressure of 25 cmH2O using 10% neutral buffered formalin. The whole lung was excised and transferred to formalin for 24 h. After fixation, the lung was transferred to 70% ethanol. Paraffin embedding, sectioning and staining with haematoxylin and eosin was performed at Seventh Wave Laboratories. Images were acquired with a Nikon E800, Plan Fluor ×10, CFIUQ (×10) at a total magnification of ×100 by QImage EXI Aqua camera (QImaging) and analysed with Image-Pro® v7.0 (Media Cybernetics).

**Histological quantification of mast cell numbers in lung tissue**

Lungs were filled with Carnoy’s fixative (60% ethanol, 30% chloroform and 10% acetic acid) under a pressure of 25 cmH2O, placed in Carnoy’s fixative for 5 h and then put in 70% ethanol. The left inferior lobe was cut in the transverse plane under the hilum of the lung. The anterior part of the lobe was dehydrated and embedded in paraffin ensuring a cross-sectional orientation and cut into 5 μm sections. To visualize mast cells, sections were deparaffinized and stained with Astra Blue (Sigma–Aldrich). Mast cell numbers were also quantified on one section from each animal. Mast cell numbers were measured using Image-Pro®.

**Statistical analyses**

Airway resistance was measured either as a maximal peak or AUC (area under the curve). To investigate the kinetics of EAR, time to peak airway resistance was analysed. To detect significant differences between groups, a one-way ANOVA followed by Bonferroni’s post-hoc test or Student’s t test respectively was applied using the Graph Pad Prism 5 software. Results of P < 0.05 were considered statistically different.

**RESULTS**

**EAR is present in a guinea-pig model of allergic inflammation that displays AHR**

To verify that the protocol generated AHR, naïve and OVA-sensitized guinea-pigs were challenged with aerosolized Mch. Sensitized guinea-pigs demonstrated significant AHR at 0.3 and 1 mg/ml of Mch when compared with naïve animals (Figure 2A). To measure EAR in the same model, guinea-pigs were challenged with OVA and lung function was monitored. As expected, OVA had no effect on airway resistance in naïve animals (Figures 2B and 2C). In contrast, an EAR with a marked increase of airway resistance was observed in sensitized guinea-pigs (Figures 2B and 2C). Moreover, the kinetics of the OVA and Mch responses were different from each other (Figure 2B). Upon Mch challenge, airway resistance developed instantaneously and then rapidly declined. Upon OVA challenge, it took approximately 3 min to achieve maximum airway resistance, and the response then waned slowly over 20 min (Figure 2B). This response to antigen challenge was shown to be highly reproducible (Figure 2C), and subsequent pharmacological experiments were performed to elucidate the mediators involved in the guinea-pig EAR.

**Pharmacological inhibition of histamine, prostanoids or leukotrienes as a single treatment has minor effects on EAR**

To evaluate the contribution of specific mast cell mediators released during EAR, histamine was antagonized with the histamine H1 receptor-antagonist pyrilamine, CysLT responses were antagonized with the CysLT1 receptor antagonist montelukast and the biosynthesis of prostanoids was inhibited by indomethacin. Treatment with a single inhibitor did not reduce the peak resistance or the overall EAR response (Table 1; Figure 3). On the other hand, we could identify kinetic differences in the response to the antigen challenge. Pyrilamine significantly delayed the time to peak response (Figure 3A).
Table 1 EAR after provocation with OVA aerosol in the absence and presence of treatments with the histamine H$_2$ receptor antagonist pyrilamine, the COX-inhibitor indomethacin and/or the CysLT$_1$ receptor antagonist montelukast

Values are expressed as mean ± S.E.M. Data are analysed using ANOVA followed by Dunnet’s post-test: *P < 0.05; **P < 0.01 and ***P < 0.001. OVA, ovalbumin-sensitized and -challenged guinea-pigs; P, pyrilamine; I, indomethacin; M, montelukast; N/C, not calculated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Peak (cmH$_2$O s/ml)</th>
<th>AUC</th>
<th>Time to peak airway resistance (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>5</td>
<td>0.15 ± 0.02</td>
<td>3.0 ± 0.3</td>
<td>N/C</td>
</tr>
<tr>
<td>OVA</td>
<td>23</td>
<td>4.66 ± 0.56***</td>
<td>40.1 ± 4.1***</td>
<td>3.11 ± 0.29</td>
</tr>
<tr>
<td>OVA + P</td>
<td>5</td>
<td>3.13 ± 0.46</td>
<td>38.2 ± 5.8</td>
<td>6.70 ± 0.73**</td>
</tr>
<tr>
<td>OVA + I</td>
<td>5</td>
<td>4.23 ± 1.23</td>
<td>41.4 ± 8.2</td>
<td>3.12 ± 0.52</td>
</tr>
<tr>
<td>OVA + M</td>
<td>5</td>
<td>4.19 ± 0.71</td>
<td>37.0 ± 3.4</td>
<td>2.40 ± 0.86</td>
</tr>
<tr>
<td>OVA + P + I</td>
<td>5</td>
<td>2.94 ± 0.76</td>
<td>30.8 ± 5.6</td>
<td>6.90 ± 1.33**</td>
</tr>
<tr>
<td>OVA + P + M</td>
<td>5</td>
<td>1.52 ± 0.36*</td>
<td>19.2 ± 3.7*</td>
<td>7.70 ± 1.06***</td>
</tr>
<tr>
<td>OVA + M + I</td>
<td>5</td>
<td>4.33 ± 1.01</td>
<td>39.7 ± 5.9</td>
<td>2.63 ± 0.97</td>
</tr>
<tr>
<td>OVA + P + M + I</td>
<td>8</td>
<td>1.42 ± 0.33**</td>
<td>14.9 ± 2.2***</td>
<td>9.63 ± 0.97***</td>
</tr>
</tbody>
</table>

Figure 3 EAR after provocation with OVA aerosol in the absence and presence of single compound treatment with (A) the histamine H$_2$ receptor antagonist pyrilamine, (B) the COX-inhibitor indomethacin and (C) the CysLT$_1$ receptor antagonist montelukast

Results show (left to right) overall airway resistance (Raw; mean), airway resistance expressed as the AUC (mean ± S.E.M.), and time to peak resistance (mean ± S.E.M.). Data from airway resistance are analysed by ANOVA followed by Dunnet’s post-test and data from time to peak airway resistance are analysed with unpaired two-tailed Student’s t test: ***P < 0.001; NS, non-significant at alpha = 0.05.
Early allergic bronchoconstriction in guinea-pigs

Figure 4 EAR after provocation with OVA aerosol in absence and presence of dual compound treatment with (A) pyrilamine and indomethacin, (B) pyrilamine and montelukast and (C) montelukast and indomethacin

Results show (left to right) overall airway resistance (Raw; mean), airway resistance expressed as the AUC (mean ± S.E.M.), and time to peak resistance (mean ± S.E.M.). Data from airway resistance are analysed by ANOVA followed by Dunnet’s post-test and data from time to peak airway resistance are analysed with unpaired two-tailed Student’s t test: *P < 0.05, **P < 0.01 and ***P < 0.001; NS, non-significant at alpha = 0.05.

with a tendency for decreased response, whereas indomethacin (Figure 3B) and montelukast (Figure 3C) showed a trend towards increased amplitude of the peak of the allergen-induced bronchoconstriction (Figure 3C).

Pyrilamine in combination with either montelukast or indomethacin, but not montelukast in combination with indomethacin, reduces EAR

As single inhibitor treatment was ineffective in blunting the EAR, the next step was to assess EAR when pyrilamine, montelukast and indomethacin were given in combination with each other. Dual treatment with pyrilamine and indomethacin showed a trend of decreased EAR (Table 1; Figure 4A), whereas treatment with pyrilamine and montelukast significantly decreased EAR, as measured by the peak resistance and AUC of the response (Figure 4B). However, the combination with montelukast and indomethacin did not reduce allergen-induced bronchoconstriction (Figure 4C). The dual treatments of pyrilamine and montelukast as well as pyrilamine and indomethacin also increased the time to peak of airway resistance (Figures 4A–4C).

The triple combination treatment with pyrilamine, indomethacin and montelukast profoundly inhibits the EAR

As a significant portion of the antigen-induced increase in airway resistance still remained after dual treatment, we next tested the efficacy of a triple compound combination. When guinea-pigs were treated with all three inhibitors, a significant reduction and a delayed peak of the allergen-induced increase of resistance was obtained (Figure 5). The reduction was numerically greater but not statistically different when compared with the most efficient dual treatment, i.e. with pyrilamine and montelukast (t test AUC: P = 0.31) and indicated that approximately 70% of the EAR can be attenuated with this treatment strategy.
**Histological evaluation of the lung tissue**

Histological evaluation of the lung tissue confirmed the successful induction of tissue inflammation with a predominant eosinophil cell infiltrate following the sensitization and challenge protocol (Figure 6). Moreover, numerous mast cells were observed around both the larger airways and vessels (Figure 7). The mast cells were also present around smaller bronchi and vessels, although not as densely as adjacent to the larger airways and vessels (Table 2). Mast cells were mainly found in the submucosal layer and were evenly scattered throughout the whole parenchyma. There were no differences in number of mast cells in either of the compartments analysed when comparing mast cell distribution from sensitized and challenged animals with naïve controls.

**DISCUSSION**

We used a short antigen sensitization and challenge protocol and characterized a guinea-pig model with marked AHR and a pronounced increase in lung resistance during the EAR. Through a systematic and stepwise analysis of pharmacological interventions we documented that combined treatment with antagonists to histamine H1 and CysLT1 receptors block the main part of the EAR in our guinea-pig in vivo model. Histological evaluation showed an induction of lung tissue inflammation with an infiltration of mainly eosinophils and a distribution of mast cells in both the proximal and distal parts of the lung. The data replicate and extend findings in allergen-induced bronchoconstriction of
Early allergic bronchoconstriction in guinea-pigs

![Figure 7 Astra Blue-stained guinea-pig lung sections for mast cell detection in different compartments of the lung (representative sections from control animals)](image)

The Astra Blue dye stains sulfated mucopolysaccharides like heparin in mast cell granules. Since cartilage is rich in proteoglycans this structure also stains with Astra Blue. The evaluation represents slides from five animals.

Asthmatics and suggest that the guinea-pig has advantages over mice for asthma research on mediator mechanisms.

EAR is triggered by an instant release and secretion of mast cell mediators that contract the airway smooth muscle. When selectively inhibiting histamine, CysLT and prostanoids, known to have strong contractile effects in vitro on isolated guinea-pig airways [18,19], no decrease of the maximal or total effect was obtained. This inability to antagonize a single pathway (histamine H1 or CysLT1 receptors) has also been shown in contractions in isolated guinea-pig lung parenchyma [14]. However, the kinetics of the response to allergen were different when the animals were treated with either pyrilamine or montelukast. Pyrilamine increased the time to the initial peak, indicating that histamine is the main mediator of the initial component of the response, whereas CysLTs contributed to the sustained phase of the response following the peak, in line with CysLTs being the slow reacting substance [20]. A similar pattern of action for histamine and CysLTs has been described during allergen-induced contractions in isolated guinea-pig lung parenchyma [14] and in IgE-dependent contractions of isolated human bronchi [10,21]. Taken together, these kinetic differences support that the different mediators contribute to distinct parts of the reaction, and are in agreement with the differences in their pharmacodynamic effects on smooth muscle (e.g. histamine short-lived rapid response and CysLTs slow in onset long-lived response).

In contrast to the small effect seen with one antagonist class, a greater decrease of the allergen-induced airway obstruction was found with combined antagonism of pyrilamine together with either montelukast or indomethacin, although the latter combination was less effective. This contrast with the responses in the guinea-pig perfused and ventilated lung, where the combination of histamine H1 receptor antagonism with COX-inhibition led to a stronger effect than combining a histamine H1 receptor antagonist with montelukast [22]. Thus, the effect of inhibiting the COX-pathway seems to be less pronounced in vivo, which is probably because of a more complex release of contractile and relaxant prostanoids during airway inflammation. A strong effect of the combination of histamine H1 and CysLT1 receptor antagonism has also been described in the EAR in mild asthma [23–25]. However, in the patients, both antagonists had an effect as a single agent. This discrepancy could be explained by the dose of antigen used in the challenge. In guinea-pigs, we use a dose of OVA that provides a maximal bronchoconstrictive response, whereas a human allergen challenge for safety purposes uses a dose that provides a submaximal, yet measurable, bronchoconstrictive response (usually a 15–25% fall in lung function). Nonetheless, both CysLTs and histamine are dominant mediators in EAR in both this guinea-pig model and the bronchial allergen challenge in asthmatic subjects. This study also highlighted that a combination of antagonists, targeting both histamine and leukotrienes, would be more effective at treating the bronchoconstriction associated with allergen challenge.

The effects of global inhibition of PGs (prostaglandins) with the COX inhibitor indomethacin were modest in this study. Thus, the combination of indomethacin with histamine H1 receptor or with histamine H1 receptor antagonist and CysLT1 receptor antagonist further inhibited the bronchoconstrictive response. This triple combination has been shown to be more effective in two guinea-pig in vitro systems using parenchymal strips and isolated

<table>
<thead>
<tr>
<th>Table 2 Number of mast cells in different anatomical compartments of the guinea-pig lung</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell type</strong></td>
</tr>
<tr>
<td><strong>Diameter (mm)</strong></td>
</tr>
<tr>
<td>Mast cells/mm² (n)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E.M. (n = 5). *Mast cells/mm².
perfused and ventilated lung [22]. Both PGD₂ and TXA₂, which are released during antigen challenge, induce strong contractions of parenchymal strips through activation of the TP receptor [13]. Conversely, PGE₂, which is also secreted during antigen challenge in guinea-pigs [22], induces strong relaxation through EP₂ receptors in guinea-pig [26]. Taking this complexity into consideration, as specific prostanoids have different and opposing effects, it is not surprising that the effects of indomethacin on the in vivo EAR response were less clear-cut. Further studies using selective prostanoid receptor antagonists need to be performed to study the influence of individual prostanoids in vivo. It should be taken into account that antagonists against the TP receptor would be most suitable as triple-therapy to effectively block the mast cell induced contractions.

In this study, about 30% of the bronchoconstrictive response was not reversed by histamine H₁ and CysLT₁ receptor antagonism together with inhibition of the COX pathway. A similar result was also reported in an in vitro system using lung parenchymal strips [14], whereas in other in vitro models, such as the isolated perfused and ventilated lung as well as precision cut lung slice, complete inhibition of allergen-induced effects was shown with the same triple combination [22,27]. Based on the previous reports [15–17], we used high doses of each class of inhibitor, so the probability of incomplete inhibition is low. From experiments in the guinea-pig lung parenchyma it was concluded that serotonin, platelet activating factor, adrenergic substances, substance P and lipid mediators not formed through the COX-pathway were involved in the EAR. However, it is possible that a more complex release of mediators during airway inflammation and an influence of an intact nervous system are present in this in vivo model, as well as the CysLT₂ receptor that has been reported to induce contraction in guinea-pig airways [18], are responsible for the residual effect.

Previous studies have utilized single- or double-chambered plethysmographs to show allergen-induced responses in the guinea-pig [28–31]. In this study, we used the forced-oscillation technique to demonstrate a prominent increase in lung resistance during EAR [32]. Although guinea-pigs are not inbred like mice, the use of this technique was able to provide highly reproducible lung function data across multiple experiments.

In the present study, we found mast cells located along both large and small airways, around blood vessels and in the parenchyma of the guinea-pig lung. This is similar to the mast cell distribution in human lungs [33] and markedly contrasts with the low number and proximal location of mast cells in mice [4,5]. Thus, data from this study support the idea that the qualitatively and quantitatively different responses to antigen challenge of guinea-pigs and mice are explained by the low number and different localization of mast cells. In addition, the contractile responses are mediated by all three classes of mediators in guinea-pigs versus mice.

Although this study demonstrated the effect on EAR, our findings are also relevant for LAR (late allergic reaction), as studies in asthmatic subjects have shown that the mediators of bronchoconstriction are the same during the LAR [23,25]. As LAR has been suggested to be even more important than EAR for both the inflammation and AHR in asthma [34], several groups has been able to develop guinea-pig models that elicit LAR [31,35,36]. This is something that is planned in our future studies.

Combination treatment has been useful in asthma when using long-acting β₂-adrenoceptor agonists together with corticosteroids. The use of CysLT₁ antagonist as a treatment has also shown benefit to asthma both as therapy alone or together with the above-mentioned combination treatment [20]. However, although histamine H₁ receptor antagonists are extensively used in treating allergic disorders such as allergic rhinitis and urticaria, they have shown limited benefit in the treatment of asthma [37]. The reasons for this is not known but may be that several mediators needed to be blocked simultaneously to achieve an effect on bronchoconstriction as described in the present study. This may also be the explanation why marginal effects are sometimes seen in studies with CysLT₁ antagonists. Indeed, a small short-term study found that the combination of montelukast and the histamine H₁ receptor antagonist loratadine was more effective in treatment of asthma than montelukast alone [38]. Further studies are needed to elucidate the role of prostanoids during allergen challenge such as a TP receptor antagonist.

**CLINICAL PERSPECTIVES**

- One feature of allergic asthma, the EAR (early allergic reaction), is not present in the commonly used mouse models and therefore we investigated the mediators involved in EAR in a guinea-pig in vivo model of allergic airway inflammation.
- In the present study, we show that, in particular, histamine and CysLTs are released during EAR in guinea-pig. Our findings highlight the significance of specific combination treatment to improve inhibition of EAR.
- We propose that this model is useful and predictive for studies of human mast cell-dependent responses and may serve as a pre-clinical model of human allergen challenge studies.

**AUTHOR CONTRIBUTION**

Jason Riley, Barbara Fuchs, Sven-Erik Dahlén, Lars Karlsson, Navin Rao and Mikael Adner participated in the research design; Jason Riley, Barbara Fuchs and Lisa Sjöberg conducted the experiments; Jason Riley, Barbara Fuchs, Lisa Sjöberg, Sven-Erik Dahlén, Navin Rao and Mikael Adner performed the data analysis; Jason Riley, Barbara Fuchs, Gunnar Nilsson, Sven-Erik Dahlén, Navin Rao and Mikael Adner wrote or contributed to the writing of the paper.

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