Characterizing effects of glucocorticoids and nitric oxide in airway inflammation in a murine in vivo model

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Abstract

Asthma is a common complex disease affecting one in twenty adults and one in ten children. It is characterized by airway hyperresponsiveness, inflammation of the airways, and infiltration of inflammatory cells into the airways. Current guidelines of asthma therapy have focused on the use of anti-inflammatory therapy, particularly inhaled glucocorticoids. Glucocorticoids are the most potent anti-inflammatory agents. One of the major mechanisms by which glucocorticoids act in asthma is by reducing airway inflammation and immune activation. The main purpose of this study was to investigate if glucocorticoids could blunt the airway inflammation in a murine asthma model. Another aim was to investigate if inhaled nitric oxide could attenuate the airway reactivity in mice with airway inflammation. Nitric oxide may have beneficial effects on pulmonary function in asthmatic patients.

The airway inflammation was induced in mice with a method relevant to human airway inflammation. The degree of bronchial reactivity and inflammation changes was assessed by measuring lung mechanics. Bronchioalveolar lavage was performed after the experiment and used as marker for infiltration of inflammatory cells and as measurement of inflammation. Lung mechanics was assessed by using an animal ventilator (FlexiVent®, Scireq Inc.). In this study resistance and compliance were measured.

An allergen (ovalbumin) model was used to sensitize and induce inflammation in the airways of female BALB/C (inbred mouse strain) mice. This was done by intraperitoneal injections and nebulizations of ovalbumin via the airways. Glucocorticoids were administrated before each allergen challenge and on the experiment day. Control groups were administrated phosphate buffer saline (PBS). The effect of inhaled nitric oxide on the airway inflammation was investigated by administration of nitric oxide before and during the lung mechanic experiment.

The results of this study showed that the protocol for allergen challenge resulted in inflammation of the airways. Mice challenged to allergen had significantly higher resistance, reduction in compliance and increased total cell count in bronchoalveolar lavage. Administration of glucocorticoids resulted in a significantly reduced
bronchoconstriction in the central airways; however it did not have any effect on more peripheral parts of the lung as measured by compliance. The results from inhaled nitric oxide showed that it did not have any effect in this model, which may be due to technical difficulties.

In conclusion; glucocorticoids have an effect on the airway resistance but whether this is limited to central airways or may include the periphery as well require thorough evaluations. The inhaled nitric oxide study needs further studies if it was appropriate right method for NO-delivery.
<table>
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<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
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<tr>
<td>AP-1</td>
<td>Activator protein-1</td>
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<tr>
<td>AR</td>
<td>Airway responsiveness</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<tr>
<td>C_RS</td>
<td>Respiratory Compliance</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>eNOS</td>
<td>Endothelial form of NOS (NOS-3)</td>
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<td>FOT</td>
<td>Forced Oscillation Technique</td>
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<td>GR</td>
<td>Glucocorticoid receptor</td>
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<td>GC</td>
<td>Glucocorticoid</td>
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<td>hsp90</td>
<td>Heat shock protein 90</td>
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<td>IgE</td>
<td>Immunoglobulin E</td>
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<tr>
<td>iNOS</td>
<td>inducible form of NOS (NOS-2)</td>
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<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
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<td>i.v.</td>
<td>intravenous</td>
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<td>MCh</td>
<td>Methacholine</td>
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<td>NF-κB</td>
<td>nuclear factor-κB</td>
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<td>nNOS</td>
<td>neural form of NOS (NOS-1)</td>
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<td>NO</td>
<td>Nitric Oxide</td>
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<td>NOS</td>
<td>Nitric Oxide Synthase</td>
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<tr>
<td>OVA</td>
<td>Ovalbumin</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PEEP</td>
<td>Positive end-expiratory pressure</td>
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<tr>
<td>PPM</td>
<td>Parts per million</td>
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<tr>
<td>R_RS</td>
<td>Respiratory resistance</td>
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<td>SC</td>
<td>SoluCortef</td>
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<td>SEM</td>
<td>Standard Error of Mean</td>
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Aims

The global aim of this project was to optimize methods to analyse the effect of glucocorticoids and to measure the effects of inhaled nitric oxide on GC in a mouse model for asthma studies.

The specific aims for this study were:

1. To induce allergen airway inflammation in mice and decide when to administrate the glucocorticoid and inhaled nitric oxide during the protocol.

2a. To investigate if glucocorticoids at different concentrations can blunt the inflammation and reduce the bronchoconstriction of the airways.

2b. To characterise the effect of glucocorticoids on the central airways during methacholine-induced bronchoconstriction and to investigate how the inflammatory cells are affected by different concentration of glucocorticoids.

3 To determine whether nitric oxide has an effect on airway inflammation during methacholine-induced bronchoconstriction and if nitric oxide has an effect on the inflammatory cells in the airways.
Background

Asthma

Asthma is an acute and chronic inflammatory disease of the airways that occurs in all age groups. It is one of the most common diseases today; around 300 million people in the world suffer from asthma and 250 000 people die from the disease every year [1]. About 10% of the population in several countries suffers from asthma [2].

Airway inflammation is a distinct feature of asthma. It is characterized by airway smooth muscle contraction (bronchospasm) that leads to wheezing, breathlessness, chest tightness and cough [3]. The inflammation of bronchial asthma involves recruitment and activation of inflammatory cells. It causes basement membrane thickening, epithelial cell loss, airway smooth muscle hypertrophy and hyperplasia [4]. Asthma is also characterized by an increased thickening of the airway wall and an increase in muscle mass and mucous glands. There is a 3- to 4-fold increase in the airway smooth muscle volume in patients with asthma compared to healthy subjects [5].

Fig 1. Airway inflammation and response to steroid [6].

Sudden symptomatic attacks of asthma may be caused by several factors such as allergens, viruses, air pollution and tobacco smoke [5]. Usually there is an interaction between genetic- and environmental factors that lead to the development of asthma. The genetic and environmental factors that may be most important in this process are poorly understood and the treatment remains problematic until better understanding of this disease develops [7].
There are two main types of asthma, allergic asthma and non-allergic asthma. Both patient groups have bronchial eosinophilia and there is a significant connection between eosinophil activation, asthma severity and bronchial hyperresponsiveness [5]. Allergic asthma is triggered by an immunoglobulin E mediated allergic reaction to an allergen and is the most common form. Non-allergic asthma is triggered by factors such as anxiety, stress, exercise, cold air, smoke etc [8, 9].

Patients with asthma that are exposed to aeroallergens get a T helper cell type-2 response and production of allergen-specific IgE. High exposure of allergens leads to mast cell degranulation and T cell activation with the release of proinflammatory mediators such as histamine, reactive oxygen species and leukotrienes. These are powerful constrictors of bronchial smooth muscles, they also induce mucous secretion and vasodilatation. This results in expression of chemokines and adhesion molecules on the airway epithelium and an influx of eosinophils into the airway mucosa and submucosa. Activated eosinophils can then induce airway hyperresponsiveness (AHR) and epithelial damage [10].

**Glucocorticoids**

Glucocorticoids (GC) are key hormones involved in modulating allergic inflammatory responses and have potent anti-inflammatory and immunosuppressive properties. They have inhibitory effects on the accumulation and activity of inflammatory cells like eosinophils, lymphocytes and macrophages [11].

As a response to physical or mental stress, the levels of cortisol, an endogenously produced GC, increase in the blood as a reaction of amplified secretion of corticotropic-releasing hormone in the hypothalamus. About 10 % of the circulating cortisol is free while the rest is circulating bound to plasma proteins like corticosteroid-binding globulin. This binding reduces the metabolic clearance rate of GCs, and because it is in a biologically inactive state it act as a buffer for GC [12].
Glucocorticoid receptor

GC are lipophilic molecules that cross the cell membrane by free diffusion [13]. In the cytoplasm they bind to and activate a specific glucocorticoid receptor (GR). This receptor is located in the cytoplasm of target cells [14]. GR belongs to the steroid thyroid/retinoic acid receptor superfamily [15]. Expression of GRs can be seen in almost all cell types but the density varies from 200 to 30,000 per cell. The highest amount of GR has been found in endothelial and epithelial cells [16]. GRs are widely distributed in the airways and are expressed on inflammatory and structural cells; this is illustrated in figure 2.

Fig 2. GRs are widely distributed in the airways and are expressed on inflammatory and structural cells [16].

In asthma, cells like eosinophils, T lymphocytes, dendritic cells, macrophages, epithelial cells, smooth muscle, endothelial cells and fibroblasts express multiple inflammatory genes. Especially epithelial cells are of importance as cellular targets for GC, because they express multiple inflammatory proteins that contribute to the inflammation of asthma [16].

Alternative transcript splicing of GR results in GRα and GRβ. GRα binds to GC while GRβ binds to DNA and acts as an inhibitor of GRα activity (it heterodimerizes with GRα) [15]. Relative amounts of GRα and GRβ can then determine the amount
of GC sensitivity. High expression of GRβ has been seen in the airways of patients with steroid-resistant asthma [16].

Inactive GR is complexed with two molecules of the heat shock protein (hsp90) chaperone complex and with the p59 protein. This complex prevents GR to enter the nucleus and bind to DNA. When a GC ligand enters the cell and bind to the complex, GR dissociates from hsp 90 and other proteins. This result in unmasking of the receptor nuclear localization signal and GR translocates to the nucleus (figure 3).

Fig 3. A GC ligand enters a cell and bind to GR in the cytosol. Dissociation of hsp 90, leading to unmasking of nuclear localization signal which translocates the GR complex to the nucleus where it binds to the DNA for transcription/inhibition of inflammatory mediators[[14].

Binding of GR to DNA is mediated by two zinc fingers on the GC molecule and by a palindromic GC response element (GRE). GRE is located on the target DNA promoter region of positively GC-regulated genes [13]. Negatively regulated genes contain a negative GRE or are inhibited by direct or indirect interference of GR with transcriptional activity of other DNA-bound transcription factors like NF-κB, AP-1, enhancer binding protein [17].

NF-κB and AP-1

In asthma there are particularly two proinflammatory transcription factors that are involved in the regulation of gene transcription; nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). NF-κB regulates many of the inflammatory genes that are abnormally expressed in asthma and is distinctly expressed in epithelial cells. This
leads to the synthesis of cytokines, adhesion molecules, chemokines, growth factors and enzymes [18]. GR interacts with NF-κB and AP-1 and prevents them from binding to DNA and following transcription of pro-inflammatory mediators. Nitric oxide is another agent that can interact with and inhibit NF-κB and AP-1 [19].

**Nitric Oxide**

Asthmatic patients have increased levels of the gas nitric oxide (NO) in their exhaled air. Exhaled NO is a sensitive marker of airway inflammation [20]. One well-documented function of NO is vasodilatation, and it may also promote plasma exudation and oedema, and facilitate cell recruitment to the tissue. NO can act as a bronchodilator as well, but the effect is weaker than on the vasculature [21].

NO can be produced by three nitric oxide synthase (NOS) enzymes nNOS (neuronal NOS), iNOS (inducible NOS) and eNOS (endothelial NOS) [22]. Through an oxygen and NADPH dependent reaction these enzymes convert L-Arginine to NO and L-Citrulline (figure 4). It has been shown that inhaled corticosteroids inhibit the expression of iNOS in patients with mild asthma but not in the case of severe asthma [20].

![Fig 4. Generation of NO.](image)

NO is a gaseous signaling molecule [22] that plays a key role in physiological regulation of airway functions [23]. NO is a small uncharged molecule that participates in many other physiological processes, like neurotransmission and host defense [24]. Because of its size and absence of charge, NO diffuses unimpeded into and out of cells and between cellular compartments [21] and it has low solubility in water at normal conditions [25].
NO has a short half-life (1-5 s) and undergoes spontaneous oxidation to the inactive metabolites nitrite (NO$_2^-$) and nitrate (NO$_3^-$) [26, 27]. When different inflammatory cells become activated by various stimuli they generate oxidants. This results in oxidative stress which is associated with high release of NO and NO metabolites, and formation of strong reactive nitrogen species. The reactive nitrogen species then amplify inflammatory processes in the airways and the lung parenchyma[23].

NO affects inflammatory cell signaling. Recent knowledge show that NO can modify residues in proteins, called S-nitrosylation, that can modify protein function and affect ion channels, receptor systems, smooth muscle cells, cell survival etc [28].

Several endogenous and exogenous molecules can induce or suppress iNOS activity. NF- κB and AP-1 are examples of molecules that induce iNOS while corticosteroids suppress the activity. Therefore it is important with an interaction between hormones during inflammation to reach an accurate expression of iNOS [29].

**Murine models of asthma**

Murine in vivo models have been developed to study airway inflammation and mechanism in asthma. It has been shown that murine models reproduce several of the human symptoms of asthma. The asthma models are very suitable for modelling traits associated with asthma although they can not model the entire asthma phenotype [30, 31]. A standard way of measuring airway hyperresponsiveness in mice, is to give them incremental doses of a bronchial constrictor agonist and then record the response of the lung to each dose [32]. Many chemical mediators (e.g. agonists) cause features associated with asthma such as bronchoconstriction, bronchial reactivity and airway leakage [33]. The agonist can either be injected or aerosolized [32]. BALB/c mice are widely used to study AHR because they produce OVA-specific IgE and eosinophilia in the bronchial lavage fluid. These mice also respond well to intravenous or inhaled methacholine (MCh) and/or acetylcholine. There are several benefits with a mouse model; mouse is a species that offers wide availability of genetically inbred strains [30, 31].
Specific background

In previous studies that are under publication, where endotoxin was used to induce acute airway inflammation, have we seen that endotoxin down regulates the glucocorticoid receptor, and administration of steroid in these cases did not blunt the inflammatory response. We have also seen that:

Inhalation of nitric oxide (NO), or the administration of NO donors can upregulate the glucocorticoid receptor (GR) in endotoxemia.

The GR is frequently found in the airway but with endotoxemia there is a down regulation of the GR also in the airways.

Inhalation of NO or NO donors in endotoxemic experimental animals respond strongly to steroid treatment with a more efficient blunting of the inflammatory response and prevention of morphological tissue change than when NO or steroids are given alone. A possibility is that NO can effect GR through S-nitrosylation.

The global aim of this project was to optimize methods to analyse the effect of glucocorticoids and to measure the effects of inhaled nitric oxide on GC in a mouse model for asthma studies.
Materials and Methods

Lung Mechanics

Lung mechanics was calculated by airway pressure and gas flow signals that was recorded with a small animal ventilator (FlexiVent®, Scireq, Montreal, PQ, Canada). The FlexiVent is a computer-controlled piston pump that functions as a conventional ventilator where parameters such as airway resistance and airway compliance can be measured [34]. The perturbations were measured by assuming a single-compartment linear model at a sinusoidal frequency of 2.5 Hz every 8 breaths for 3.5 min after each methacholine (MCh) dose. Compliance was measured before each dose of MCh.

Resistance

This parameter reflects not only central airway resistance, but is also influenced by the lung periphery [35]. An increase in resistance illustrates narrowing of the conducting airways and alterations in the lung periphery. Resistance is defined as pressure divided by flow (P/V). The resistance to breathing is often divided into three components: airway resistance, lung tissue resistance and chest wall resistance. Lung resistance is the sum of airway and lung tissue resistance [36]. Figure 5 illustrate a typical dose response curve to methacholine, were increasing doses of MCh give an increase in resistance.

![Fig 5. A typical methacholine dose response curve, (FlexiVent®).](image)
Compliance

Respiratory compliance is reduced in patients with asthma, and may reflect many events such as, tissue edema and increased airway stiffness. Such stiffness occurs when collagen fibers and other elastic elements are increased in the subepithelial reticular layer of the airway [37].

Compliance is a static measure of lung and chest recoil and is expressed as a change in lung volume per unit change in airway pressure e.g. litres/cmH₂O. It may be thought of as the inverse of stiffness; i.e. an increase in stiffness leads to a reduction in compliance.

Animals

Female BALB/c mice (Mulle, Denmark), weighing 18.1±0.2 g, were used in this study. Food and water were provided ad libitum. Their care and the experimental protocols were approved by the Regional Ethics Committee on Animal Experiments in Uppsala, Sweden (C86/5).

Allergen airway inflammation

Mice were sensitized by 200 µl intraperitoneal injection of chicken egg albumin 10µg, (ovalbumin OVA, Sigma) at day 0 and day 7.

Mice were placed in a plastic chamber and the inflammation was induced by nebulization of phosphate-buffered saline (PBS, controls) or OVA via airways for 30 minutes on days 14, 15 and 16. OVA was mixed with PBS to attain a 1% OVA concentration. See figure 6.

![Figure 6: OVA/PBS protocol for sensitization and challenge of the mice.](image-url)
Preparation of animals

The animals were anesthetized by i.p. injection of pentobarbital sodium (100 mg/kg, Apoteket, Sweden) before the experiment at day 17. This was confirmed by the absence of response to paw pitch. Mice were placed on a 37 °C-heating pad to keep body temperature constant during the experiment. A tracheostomy was performed and a metal endotracheal tube was inserted into the trachea. Mice were mechanically ventilated with the FlexiVent®, figure 7. Tidal volume was set to 12 mL/kg bodyweight at a frequency of 150 breaths per minute. When ventilation was established bilateral thoracotomies were performed in the chest wall so that pleural pressure would equal atmospheric pressure. Positive end-expiratory pressure (PEEP) of 3 cmH₂O was generated by submerging the expiratory line (3 cm) into a water container.

![Fig 7. An illustrated picture over the FlexiVent® system.](image)

The lateral tail vein was cannulated for i.v. injections. The airway responsiveness was assessed by increasing doses (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg) of methacholine (MCh, Sigma), every 4 min. Dose-response curves were obtained by plotting peak resistance as a function of MCh dose and curves for analyzing compliance were obtained by plotting compliance as a function of treatment.

Bronchoalveolar lavage, Blood and Lungs

Bronchoalveolar lavage (BAL) was performed directly after the experiment. The lungs where lavaged 3 times via the tracheal tube with 1 ml PBS + EDTA. The BAL fluid was immediately centrifuged for 10 min, 4 °C at 1200 rpm. The supernatant was removed and stored in -70 °C for future studies. Cell pellets were resuspended in 100
μl red blood cell lysis buffer for 2 min, diluted in 1 ml PBS and centrifuged for 10 min, 4 °C at 1200 rpm. Pellets were resuspended in 1 ml PBS and the numbers of cells were counted (cells/mL).

Directly after BAL, blood was obtained via cardiac puncture and cells were counted. The rest of the blood was centrifuged for 5 min at 5000 rpm, serum was removed and stored in -70 °C. The lungs were removed, directly snap-frozen in liquid nitrogen and stored in -70 °C. Blood and lungs were stored for further investigations.

**Statistical analysis**

Results are presented as mean ± standard error of mean (SEM). A statistical result with p<0.05 was considered to be significant. In the graphs, significances are marked as follows;

| *  p<0.05 | ** p<0.01 | *** p<0.0001 |

Statistical analysis was performed with GraphPad Prism (version 4.0 GraphPad software Inc, San Diego, CA, USA). Statistical significance between and within groups were assessed by parametric methods using analysis of variance one-way or two-way (ANOVA) and Bonferroni post-test when appropriate. Student’s t-test was used to analyse cell counts in BAL and blood between two groups.
Study design

Study 1. Airway inflammation

Airway inflammation sensitization and challenge as previously described for OVA or PBS.

Study 2. The effect of glucocorticoids on the airway inflammation

Mice were treated with different doses of corticosteroid (SoluCortef). Mice were divided into four groups; three groups that were given different doses of SoluCortef (25, 37.5 and 50 mg/kg) and one group was given PBS (control). Mice were given a 200 µl i.p. injection of SoluCortef/PBS day 14, 15, 16 and one hour before the experiment, day 17. See table 1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>SoluCortef 25</th>
<th>SoluCortef 37.5</th>
<th>SoluCortef 50</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>OVA ip</td>
<td>OVA ip</td>
<td>OVA ip</td>
<td>OVA ip</td>
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<tr>
<td>Day 7</td>
<td>OVA ip</td>
<td>OVA ip</td>
<td>OVA ip</td>
<td>OVA ip</td>
</tr>
<tr>
<td>Day 14</td>
<td>PBS ip before PBS neb</td>
<td>Dose 25 ip before 1% OVA neb</td>
<td>Dose 37.5 ip before 1% OVA neb</td>
<td>Dose 50 ip before 1% OVA neb</td>
</tr>
<tr>
<td>Day 15</td>
<td>PBS ip before PBS neb</td>
<td>Dose 25 ip before 1% OVA neb</td>
<td>Dose 37.5 ip before 1% OVA neb</td>
<td>Dose 50 ip before 1% OVA neb</td>
</tr>
<tr>
<td>Day 16</td>
<td>PBS ip before PBS neb</td>
<td>Dose 25 ip before 1% OVA neb</td>
<td>Dose 37.5 ip before 1% OVA neb</td>
<td>Dose 50 ip before 1% OVA neb</td>
</tr>
<tr>
<td>Day 17</td>
<td>PBS ip 1h before Exp</td>
<td>Dose 25 ip 1h before Exp</td>
<td>Dose 37.5 ip 1h before Exp</td>
<td>Dose 50 ip 1h before Exp</td>
</tr>
</tbody>
</table>

Table 1. Glucocorticoid protocol for different doses of SoluCortef.

Study 3. The effects of inhaled Nitric Oxide

Mice were treated with inhaled nitric oxide, 40 parts per million, 15 minutes before and during the experiment on day 17. Mice were divided into four groups; one OVA challenged group and one PBS challenged group that received inhaled NO 15 minutes before and during the experiment and two control groups, OVA and PBS, which did not receive any inhaled NO.
Results

Study 1. Airway inflammation – physiological and cellular effects

The protocol for the allergen challenge resulted in inflammation of the airways. The OVA challenged mice had a significantly higher airway resistance as compared to the control group (PBS), (P<0.0001). OVA-challenged mice had higher resistance compared to PBS challenged mice at all doses above 0.1 mg/kg. See figure 8.

In OVA challenged mice, administration of MCh induced a slightly reduction in compliance as compared to PBS mice. There were no significance within the groups, see figure 9.

![Fig 8. MCh dose-response curves are shown for OVA and PBS challenged mice (mean± SEM).](image)

![Fig 9. Dose-response curve to MCh. Compliance was lower in the OVA challenged mice than in mice treated with PBS. There were no significance within the groups.](image)
The total cell count in BAL fluid was increased in mice that were OVA-challenged as compared to control (P<0.0002). See figure 10.

![Fig 10. Total number of cells in BAL fluid. Significant higher TCC in OVA challenged mice (P<0.0002)](image)

**Study 2. The effect of glucocorticoids on the airway inflammation**

*Physiological effects*

There was a significant difference in MCh-induced airway reactivity between mice treated with SoluCortef and control mice (ANOVA, P<0.0001). SoluCortef decreased the reactivity at the highest dose of MCh from $6.39\pm0.49$ cmH$_2$O/mL/s to $1.85\pm0.32$ cmH$_2$O/mL/s with dose 50 of SoluCortef, (P<0.0001). There were significant differences between the three groups which were treated with different doses of SoluCortef. Dose 50 resulted in a greater reduction in the reactivity at the higher doses of MCh (1 mg/kg and 3 mg/kg) as compared to dose 25, (p<0.0001). There were no differences between dos 25 and 37.5. See figure 11.
Fig 11a. Dose-response curve to increasing tail-vein injection of MCh. Significant difference in airway responsiveness between the control group (PBS) and the three groups treated with SoluCortef (p<0.0001).

There were no significant differences in compliance between the groups treated with SoluCortef and the control group. See figure 12.

Cellular effects

The total cell count in BAL fluid was increased in mice that were treated with PBS (P= 0.005). There was no significant difference in the number of cells between the three groups treated with SoluCortef. See figure 13.
The total cell count in blood was only significant between dose 50 and the control group which was given PBS, (P=0.038). See figure 14.
Study 3. The effects of inhaled Nitric Oxide

Physiological effects of NO

There was significance between the OVA and the PBS challenged groups. In both groups, NO did not have any effect against MCh-induced bronchoconstriction. See figure 15.

In PBS treated groups, NO administration induced a significant reduction in airway compliance (P<0.0001). There were no significant differences in the OVA treated group during NO administration. See figure 16.
Discussion

The mouse is widely used for in vivo asthma models, but what do mouse models tell us about human asthma? Today we have an asthma model that resembles asthmatic inflammation. The mouse offers good models for understanding cellular and mediator pathways in airway inflammation. Better insights into the complex mechanisms will lead to better ways to control airway inflammation and improve treatment of patients. This study focused on the effect of glucocorticoid and the effect of inhaled nitric oxide on GC on airway inflammation.

Study 1. Airway inflammation

The first aim of this study was to induce airway inflammation and according to the results, the protocol for inducing inflammation in mice was found to cause physiological and cellular effects in the lung. The physiological effect was seen as an increased airway hyperresponsiveness to methacholine. The cellular effect was an increased infiltration of cells into the airways. These findings confirm previous studies [11, 38] that OVA sensitization and challenge of mice induces airway inflammation and recruitment of cells into the lung.

Study 2. The effect of glucocorticoids on the airway inflammation

The aim of study two was to decide when to administrate glucocorticoids. Our research group has previously studied the effect of GC on pigs with airway inflammation, therefore we used the corticosteroid (SoluCortef). SoluCortef was given before each OVA challenge because we wanted to inhibit the airway hyperresponsiveness and decrease the methacholine-induced bronchoconstriction and we thought the best way to achieve this was to do it at the same time point as the inflammation was induced. We also investigated if glucocorticoids can blunt the inflammation and reduce the bronchoconstriction of the airways. According to our results in this study, the administration of SoluCortef resulted in a significantly reduced bronchoconstriction in the central airways. However, GCs did not have an effect on compliance and this can be due to that GCs have an effect on airway inflammation in the more central parts as compared to the peripheral parts of the lung.
The total cell count in BAL fluid was significantly decreased in the groups that were administrated with SoluCortef compared to the control. The second aim was also to analyze if different concentrations affect the airway hyperresponsiveness differently. The various concentrations of SoluCortef resulted in different response in AHR but not a significant difference in the total cell counts. Our results from this study are in line with other studies done in mice. In a study in BALB/c mice treated with the glucocorticoid, dexamethasone, Birelli et al [39], showed that there is a dissociation between the doses of steroid (dexamethasone) needed to affect antigen-induced BAL and lung eosinophilia and that needed to affect AHR. They also showed that the dose of steroid to inhibit AHR is higher than that needed to inhibit eosinophilia. Many previous studies, suggest that AHR is not a direct consequence of eosinophilia.

A future step is to analyze the BAL samples for each concentration of steroid treatment to investigate if the steroid SoluCortef has the same effect as dexamethasone. It would be of interest to compare the number of eosinophils in relative to AHR. According to a study by Eum et al 2003 [40], glucocorticoids are potent inhibitors of AHR and their inhibitory actions on infiltration of inflammatory cells such as eosinophils into the airways are often presumed to be an important component of their therapeutic effect. As in the study done by Birelli et al 2002 they draw a conclusion that the effects of dexamethasone on AHR are unlikely to be related to alterations in eosinophilia.

A further investigation that needs to be done is to characterize the glucocorticoid receptor in these mice and determine which cells are expressing the receptor in the airways. Can SoluCortef up-regulate the receptor and to which level? When this model has been thoroughly investigated it would be of interest to investigate if inhaled NO can up regulate the GR in this model and if steroids are more efficient on blunting the inflammatory response during inhalation of NO than without NO.

**Study 3. The effects of inhaled nitric oxide**

The third aim was to determine whether nitric oxide has an effect in airway inflammation during methacholine-induced bronchoconstriction. According to our results in this study, treatment with inhaled NO did not improve the inflammation. NO showed no significant effect on airway resistance in any of the groups. In
previous studies there is evidence that NO can act as a bronchodilator but according to our results we did not see any effect of NO. It is therefore reason to suppose that in the bag containing the gas, NO was oxidized to its metabolites nitrite and nitrate. Another possibility is that the inhaled NO concentration was too low. The role of NO in airway physiology is controversial and some previous studies state that NO may be toxic to airway tissues and increases inflammation and AHR in asthmatic patients [41] whereas other studies mean that NO is non-toxic in the lung. This method needs more thorough investigation so that we can use inhaled NO to get a better insight into the complex mechanisms of airway inflammation.

**Conclusion**

The protocol for inducing inflammation in mice was found to cause physiological and cellular effects in the lung. Treatment of glucocorticoids had an effect on the methacholine-induced bronchoconstriction and can be used as a treatment to inhibit the airway hyperresponsiveness in mice. The effect of inhaled nitric oxide, and if NO can effect GC still needs further investigations.
References


