Alterations in airway mechanics, inflammatory biomarkers and lung histopathology in a rat model of allergen-induced airway inflammation

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Introduction

- The most objective indicator of asthma severity in the clinic is the measurement of reversible airway obstruction by spirometry.
- To mimic this we evaluated the effect of antigen challenge on forced expiratory volume in 100 msecs (FEV_{100}), forced vital capacity (FVC) and peak expiratory flow (PEF) together with airway cell infiltrate, BAL cytokine levels and lung histopathology in the Brown Norway rat allergic model.
- The corticosteroid budesonide was employed as a positive control.

Methods

- Male Brown Norway rats were sensitised to the antigen ovalbumin (0.5 mg chicken egg albumin) adsorbed onto 100 mg aluminium hydroxide $Al(OH)_3$ hydrate s.c.
- Fourteen days later the rats were challenged to aerosolised ovalbumin (10 mg/mL) or 0.9%w/v saline for 15 minutes by whole body exposure on one or two occasions 24 h apart.
- Oral intervention with budesonide (3 mg/kg b.i.d.) was initiated 1 h prior to challenge.
- 24 h after a single or double challenge the rats were terminally anesthetised and a forced manoeuvres procedure performed using the eSpira Forced Manoeuvres System (EMMS).

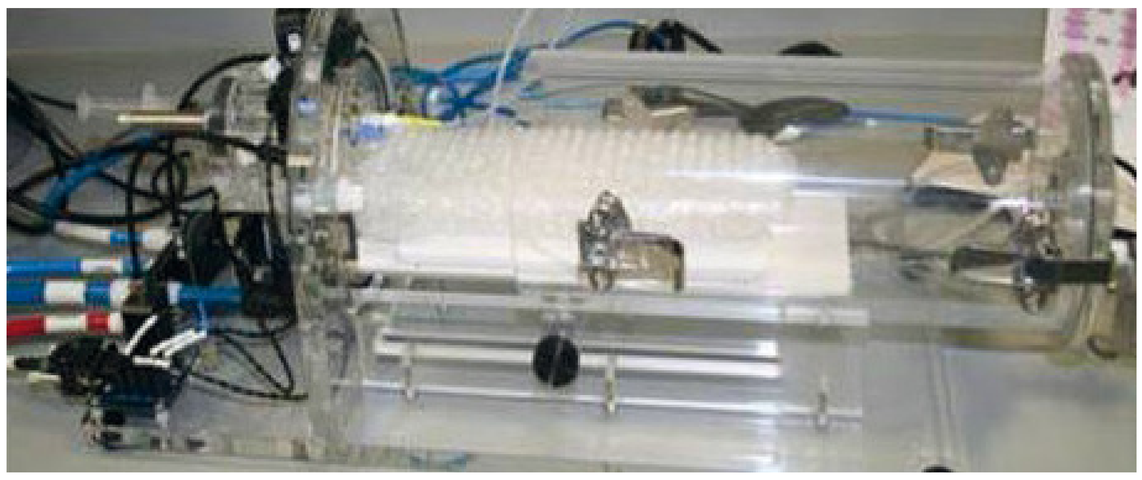

Image Source: Internal Labcorp (equipment Luminex)

- Recruitment of inflammatory cells and biomarker production was assessed in bronchoalveolar lavage fluid (BALF).
- A total and differential cell count was performed using the Sysmex-XT-Vet and BAL supernatant analysed for inflammatory cytokines using the Luminex 200.
- The lungs were insufflated with 10% neutral buffered formalin before wax embedding. Sections (5 µM) were cut and stained with H&E.

Results

- Antigen challenge caused significant reductions ($p<0.001$) of 30.2 ± 2.6% in FEV_{100}, 20.5 ± 1.7% in PEF and 43.0± 3.2% in FVC 24 h following a single challenge when compared to saline challenged animals. There was no further decline in lung function 24 h following a double ovalbumin challenge.
- Oral administration of budesonide completely reversed the decline in FEV_{100}, PEF and FVC 24 h following a single or double challenge.
- Exposure to antigen resulted in significant recruitment of eosinophils (2.13±0.60 x10^6 cells/animal), neutrophils (2.64±0.36 x10^6 cells/animal) and lymphocytes (0.53±0.05 x10^6 cells/animal) into the airway following a single challenge. Cell infiltration was further elevated (approximately 2 fold) following a second challenge.
- Animals treated with budesonide resulted in a significant ($p<0.001$) decrease in all BAL cell types following a single or double challenge.
- Antigen exposed animals exhibited significantly ($p<0.001$) increased levels of GRO-α, IL-6, MIP-1α, TNF-α and allergic challenge cytokines IL-13 and IL-5 24 h after a single or double challenge. Levels of IL-5 and IL-13 were significantly ($p<0.01$) reduced 24 h following a double challenge compared to the same time point following a single challenge.
- Budesonide treatment reduced BAL cytokine levels to similar levels recorded in the saline challenged animals.

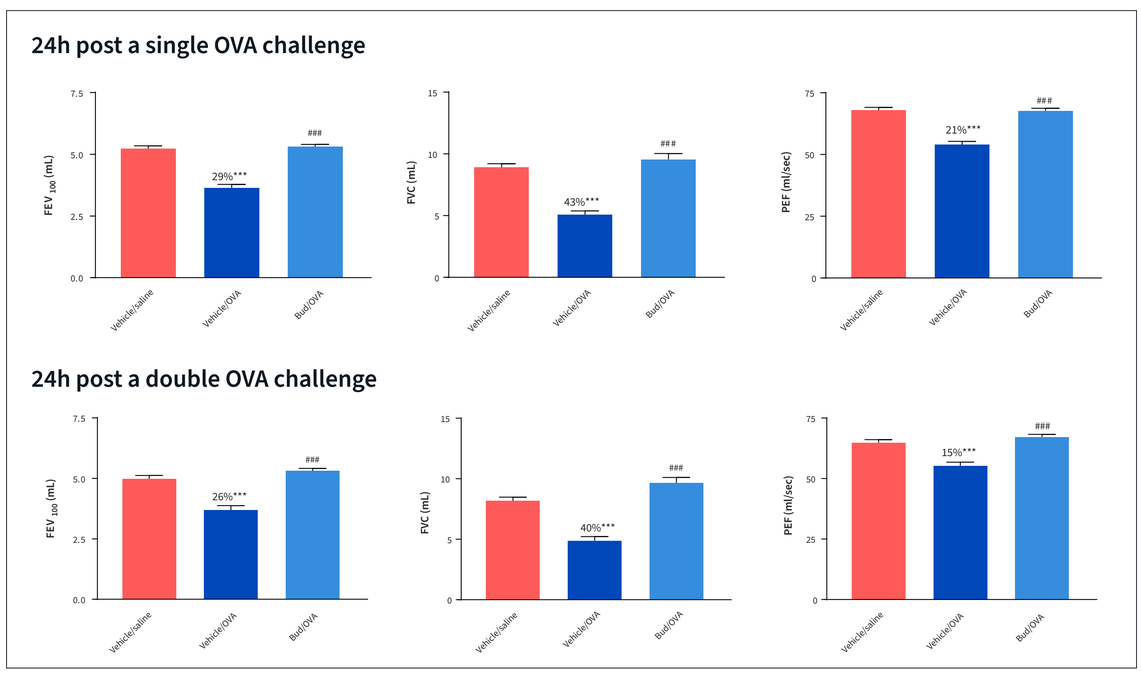

Figure 1. Effects of budesonide treatment on antigen (Ovalbumin)-induced lung function decline. Mean ± SEM (n=8 per group). ***p<0.001 when compared to the vehicle/saline treated animals. ###p<0.001 when compared to the OVA/vehicle treated animals.

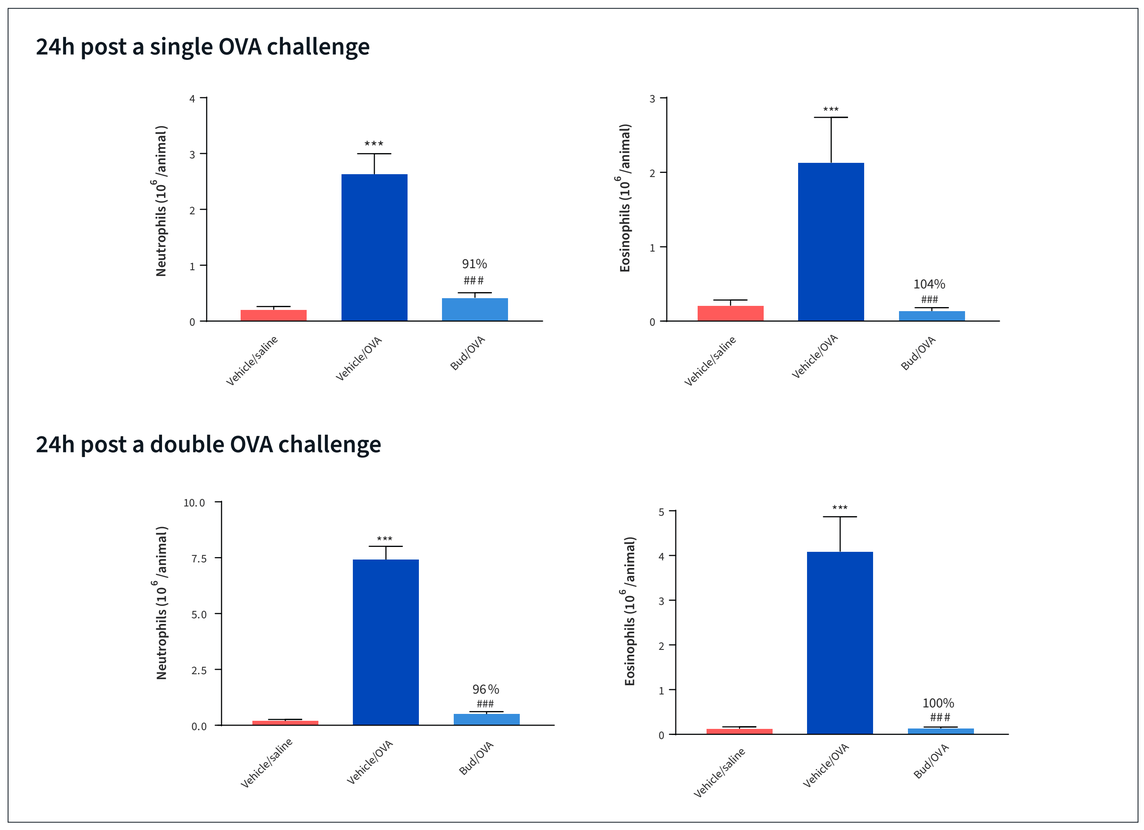

Figure 2. Effects of budesonide treatment on antigen (Ovalbumin)-induced BAL inflammatory cell infiltration. Mean ± SEM (n=8 per group). ***p<0.001 when compared to the vehicle/saline treated animals. ###p<0.001 when compared to the OVA/vehicle treated animals.

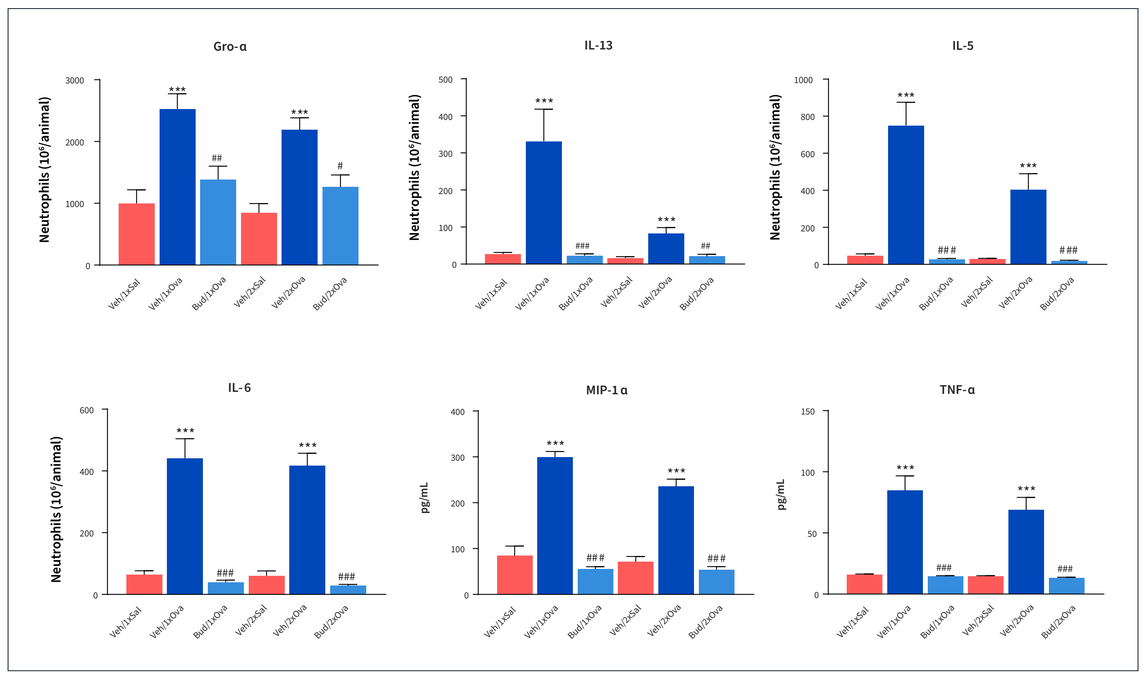

Figure 3. Effects of budesonide treatment on antigen (Ovalbumin)-induced increases in BAL Cytokines. Mean ± SEM (n=8 per group). ***p<0.001 when compared to the vehicle saline treated animals. #p<0.05, ##p<0.01, ###p<0.001 when compared to the OVA/vehicle treated animals.

Image Source: Internal Labcorp

Figure 4. Photomicrographs of representative sections of lungs taken from a. saline challenged animals, b. double OVA challenged animals and c. budesonide treated double OVA challenged animals.

Conclusion

- We have demonstrated that allergen (ovalbumin) challenge results in a reversible decline in measured FEV_{100}, PEF and FVC in a rat allergic model.
- These functional changes are concomitant with traditional markers of lung inflammation and are reversed with steroid treatment.
- This functional measurement may be a valuable tool for translating the efficacy of novel compounds from rodents to the clinic.

Bibliography

1. Underwood SL, Haddad E, Birrell MA, McCluskie K, Pecorara M, Dabrowski D, Webber SE, Foster ML, Belvisi MG. Functional characterization and biomarker identification in the Brown Norway model of allergic airway inflammation. British Journal of Pharmacology (2002) 137, 263-275.
2. Snell N and Newbold P. The clinical utility of biomarkers in asthma and COPD. Current Opinion in Pharmacology 2008, 8: 222-235.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.