Efficacy of an Inhaled PDE4 Inhibitor, GSK256066, Delivered by a Novel Dry Powder Preclinical Inhalation Delivery System in the Acute Cigarette Smoke-Induced Pulmonary Inflammation Model

D. Rodgers[1], C. Gutteridge[1], G. Paul[2], S. Moore[1], K. Meecham[1] and S. Jordan[1]
[1]Labcorp Drug Development Limited, Huntingdon, UK; [2]GlaxoSmithKline, Stevenage, UK

Introduction

- Short-term cigarette smoke (CS) exposure in mice has been shown to induce changes exhibiting similarities to cell and cell-mediated inflammation observed in patients with COPD.
- Our aim was to evaluate the efficacy of an inhaled PDE4 inhibitor, GSK256066, in a murine 4-day acute cigarette smoke model.
- GSK256066, an exceptionally high affinity and selective inhibitor of PDE4,[1] was delivered as an inhaled dry powder using a novel delivery system, a capsule-based aerosol generator (CBAG), to maximise delivery efficiency and minimise test substance requirements.

Methods

- Female C57BL/6 mice were exposed to a sub-maximal concentration of 3R4F CS (600 µg/L) or air, twice daily (1 h/exposure on each occasion) for four consecutive days using a whole-body exposure system.
- Lactose or GSK256066 (target doses 30, 300 and 1000 µg/kg) were dosed by nose-only inhalation using CBAG twice daily for 30 min, 1 h prior to the start of each CS exposure. Budesonide (300 µg/kg) and GSK256066 (200 µg/kg) were administered to additional groups of mice by the intranasal route 1 h prior to the start of CS exposure.
- The CBAG (Figure 1) was designed and co-developed by the Labcorp Drug Development inhalation scientists and engineers and inhalation scientists at GSK.[2] Principally, the capsule actuator is able to load, pierce and aerosolise the contents of individual capsules through a sequential mechanised cycle. Compressed air is used to aerosolise the contents of the capsule.
- Approximately 18 h following the final CS exposure, animals were terminated and a bronchoalveolar lavage (BAL) performed for assessment of total and differential cell counts and cytokine release.

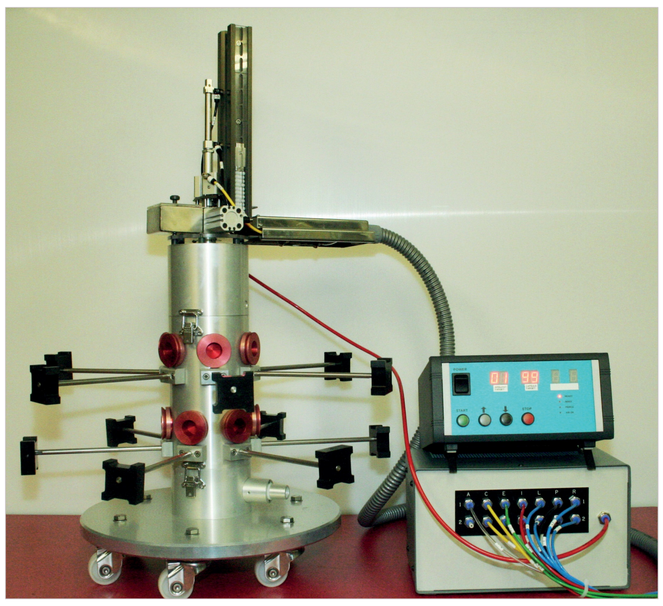

Figure 1. Capsule-based aerosol generator (CBAG).
Image source: Internal Labcorp.

Results

- GSK256066 aerosol concentrations from the inhalation exposure chambers were all within 15% of target and the particle size was within the respirable range for the mouse.
- Acute CS exposure resulted in a statistically significant ($p<0.001$) neutrophil-driven inflammatory response in the airways with elevated BAL levels of KC ($p<0.01$), MIP-2 ($p<0.05$), MCP-1 ($p<0.001$) and IL-6 ($p<0.05$) compared to lactose/air control animals.
- Exposure to increasing doses of GSK256066 at delivered doses of 34, 307 and 864 µg/kg produced a dose-dependent inhibition of BAL neutrophilia with maximal efficacy of approximately 50% at 307 and 864 µg/kg, respectively.
- BAL KC was reduced by inhaled and intranasal administration of GSK256066 and also by steroid intervention (Budesonide).
- IL-6 was significantly increased only in the inhaled GSK256066/CS-treated animals compared with vehicle/CS-treated animals (peak response at 307 µg/kg: 241 vs 17 pg/mL).
- Intranasal administration of GSK256066 (200 µg/kg) and Budesonide (300 µg/kg) did not inhibit cellular recruitment.
- The GSK256066 compound requirement to achieve a delivered dose of 864 µg/kg was 38 mg, compared to 875 mg for a standard Wright Dust feed mechanism.

Table 1. Aerosol Concentration, Particle-Size Distribution and Estimation of Inhaled Dose

Parameter	Low dose	Inter dose	High dose
Target aerosol concentration (µg/L)	0.95	9.5	31.8
Achieved aerosol concentration (µg/L)	1.0	9.3	26.2
Target dose (µg/kg)	30	300	1000
Estimated inhaled dose (µg/kg)*	33.7	307	864
Mass median aerodynamic diameter (µm)	2.8	2.3	3.1
Geometric standard deviation	2.6	3.0	2.5

*Inhaled dose calculated using AIT equation.[3]

Conclusion

- The 4-day acute CS model induces a robust inflammatory response in the murine lung. Inhaled administration of the PDE4 inhibitor GSK256066 significantly attenuated the neutrophil driven airway inflammation.
- Inhaled administration appeared relatively more efficacious than the similar intranasal dose of GSK256066, supporting the hypothesis that the more diffuse lung delivery may improve efficacy compared with the bolus intranasal delivery.
- The augmentation of CS-induced BAL IL-6 levels by inhaled GSK256066 remains unclear, but selective PDE4 inhibitors have been shown to increase IL-6 release in LPS-activated whole blood from rats.[4]
- The CBAG offers a significant cost saving in test article production over commercially available instruments as it minimises dead volume waste whilst delivering clinically relevant lung particle deposition.

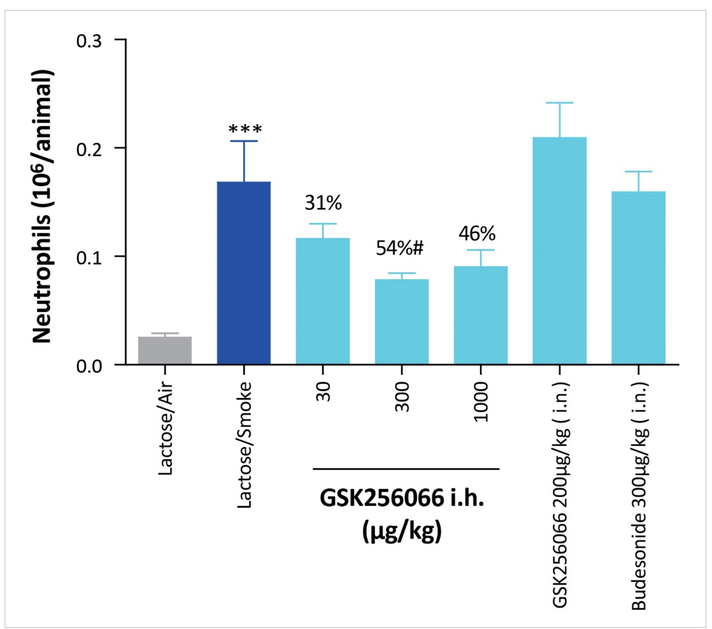

Figure 2. Effect of GSK256066 on CS-induced BAL neutrophilia (mean ± s.e.m. n=10/group).
***p<0.001 when compared to the Lactose/Air group.
# p<0.05 when compared to the Lactose/Smoke group.
Inhaled GSK256066 target doses used in figure.

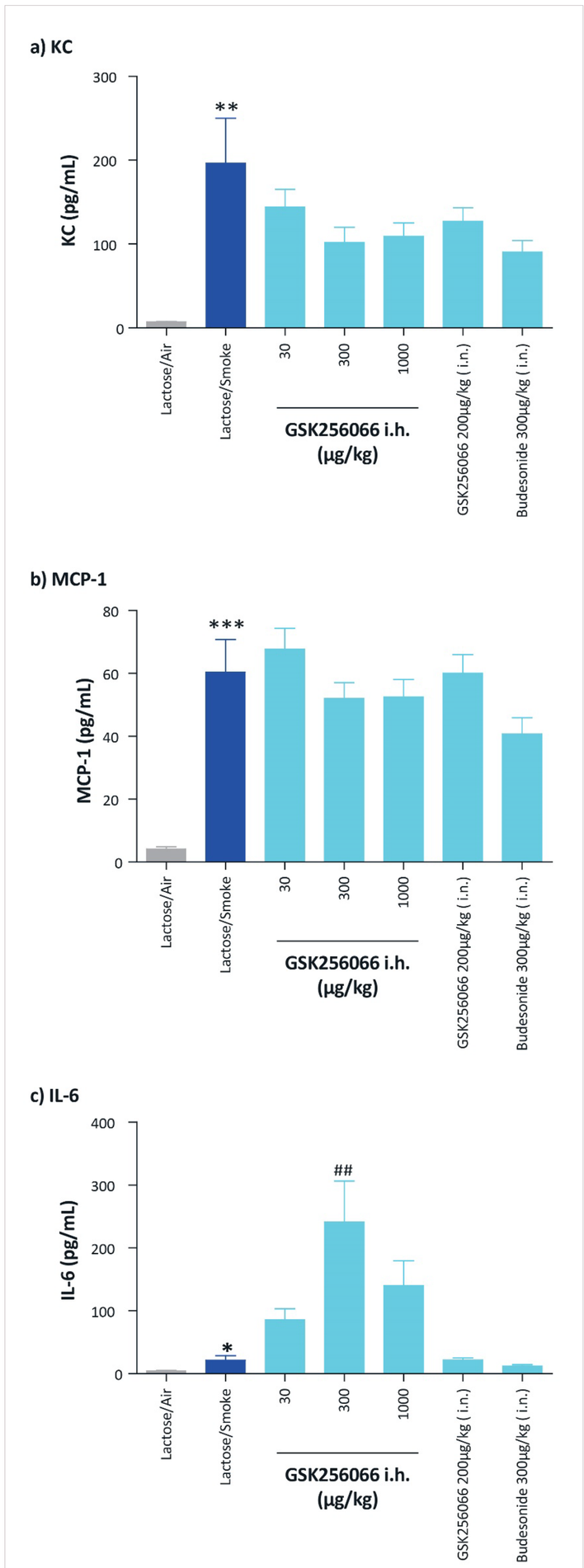

Figure 3. Effect of GSK256066 on CS-induced BAL cytokines (mean ± s.e.m. n=10/group).
***p<0.001, **p<0.01, *p<0.05 when compared to the Lactose/Air group.
p<0.01 when compared to the Lactose/Smoke group.
Inhaled GSK256066 target doses used in figure.

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.

References

1. Tralau-Stewart C.J., Williamson R.A., Nials A.T. et al. GSK256066, an Exceptionally High-Affinity and Selective Inhibitor of Phosphodiesterase 4 Suitable for Administration by Inhalation: In Vivo, Kinetic, and In Vivo Characterization, JPET, 2011, 337:145–154.
2. Paul G.R., Somers G.I., Moore S.A. and Goodway R.E. The Capsule Based Aerosol Generator – Conserving Test Article in Small Scale Inhalation Exposures, Respiratory Drug Delivery, 2012, Volume 2, 525-530.
3. Alexander D.J., Collins C.J., Coombs D.W. et al. Association of Inhalation Toxicologists (AIT) working party recommendation for standard delivered dose calculation and expression in nonclinical aerosol inhalation toxicology studies with pharmaceuticals. Inhal. Tox., 2008, 1179-1189.
4. Dietsch G.N., DiPalma C.R., Eyre R.J. et al. Characterization of the Inflammatory Response to a Highly Selective PDE4 Inhibitor in the Rat and the Identification of Biomarkers that Correlate with Toxicity. Toxicologic Pathology, 2004, 34:39-51.