The impact of dosage timing for inhaled corticosteroids in asthma: a randomised 3-way crossover trial

Ran Wang[1, 2], Robert Maidstone[1], Dave Singh [1,2,3], David Ray[4, 5], Andrew Loudon[1], Angela Simpson[1, 2] and Hannah J Durrington[1, 2]

1. Faculty of Biology, Medicine and Health, School of Biological Sciences, Division of Immunology, Immunity to Infection & Respiratory Medicine, The University of Manchester, United Kingdom.
2. NIHR Manchester Biomedical Research Unit and Manchester University NHS Foundation Trust, United Kingdom
3. Medicines Evaluation Unit, Wythenshawe, United Kingdom
4. NIHR Oxford Health Biomedical Research Centre, and NIHR, Oxford Biomedical Research Centre, John Radcliffe Hospital, Oxford, UK
5. Oxford Centre for Diabetes, Endocrinology and Metabolism, and Oxford Kavli Centre for Nanoscience Discovery, University of Oxford, Oxford, UK.

Corresponding author:

Dr Hannah J Durrington

The University of Manchester

AV Hill building

Upper Brook St

Manchester

M13 9LJ

Tel: (+44)161 275 1148

Email: Hannah.durrington@manchester.ac.uk

Section E1: Methodological Details

Study procedures

Spirometry was carried out (NDD Easy on-PC Ultrasonic Spirometer) in accordance with American Thoracic Society / European Respiratory Society (ATS/ERS) 2005 criteria and ATS Technical Statement 2017 (1, 2) All participants withheld short-acting beta2 agonist for 4 hours prior to testing. The predicted normal values used were based on the Global Lung Function Initiative predicted values (3).

Fractional exhaled nitric oxide levels were measured (NIOX VERO, Circassia, UK) in accordance with ATS/ERS 2005 criteria (4). Participants were asked to refrain from xanthine (caffeine containing food and beverages) for at least 12 hours before all study visits. Participants were asked to refrain from foods with high nitrate content for at least 6 hours and any food for at least 1 hour prior to each FeNO measurements.

Sputum inductions were performed by administering hypertonic saline (3%) through a nebuliser (NE-U17, Omron, Japan) for 5 minutes. Participants were asked to rinse their mouth to remove excess saline and saliva and blowing their nose to remove nasal secretions and were asked to powerfully huff and cough to enable them to expectorate sputum. This procedure was repeated two more times with 4% and 5% saline in 5 minutes cycles, provided the participants' lung function remained stable (< 10% fall in FEV_1). If the FEV_1 falls by 10-20%, the next cycle is performed at the same concentration as previously; if > 20% all, the induction is terminated. For the 4 AM inductions, they were performed under dim light to minimise circadian disruption.

Peak flow monitoring: all participants were trained in the use of peak flow meter and instructed to perform three measurements twice daily (i.e., in the morning between 7 AM and 9 AM, and in the evening between 7 PM and 9 PM). Participants were instructed to carry out the measurements before they take the study medication.

Inclusion and exclusion criteria:

Subjects will only be included if they meet all applicable inclusion criteria and no applicable exclusion criteria.

Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for randomisation into the study:

1. Male and female adults aged 18 to 65 years at the time of screening and with written informed consent obtained prior to any study-related procedure.

2. Subjects with a physician diagnosis of mild to moderate allergic asthma and with symptoms compatible with asthma for at least 1 year prior to screening.

3. Clinically stable asthma in the 3 months prior to screening and prior to randomisation.

4. Treatment with low to medium doses of inhaled corticosteroids with or without long acting beta agonists.

5. Asthma Control Questionnaire score of < 1.5 score at screening.

6. Body mass index (BMI) in the range of 18.0 to 33.0 kg/m2 at screening.

7. Non-smokers or ex-smokers being defined as someone who completely stopped smoking cigarettes (including e-cigarettes) for at least 12 months prior to screening and with a smoking history of less than 5- pack years.

8. Oxygen saturation ≥92% on room air at screening

9. Pre-bronchodilator spirometry FEV1 ≥65% of predicted at screening and FEV1 ≤90% of predicted during either the screening or run-in period or on Day -4, Baseline Period (Visit 2).

10. Bronchial hyperresponsiveness assessed as documented evidence of variable expiratory airflow limitation during the screening or run-in periods or in the past 2 years prior to Screening, defined as one of the following:

- ≥12% or ≥200 mL improvement in FEV1 in a bronchodilator reversibility test
- Provocative dose or concentration of methacholine resulting in a ≥20% drop in FEV1 (PD20 or PC20, respectively) of ≤ 1mg or ≤ 16 mg/mL, respectively.

11. Sputum producer stratum only (minimum of 14 of the 25 planned subjects): Able to generate an adequate sputum sample for processing (as per study reference manual) following induction with inhaled, nebulized hypertonic saline at screening

12. Subject is willing and, in the opinion of the Investigator, able to change current asthma therapy (i.e. withdraw form inhaled steroids and long acting bronchodilators; if applicable) as required by the protocol.

13. Documented allergy to at least one common allergen (i.e. cat dander, house dust mite or grass pollen) as confirmed by the skin prick test wheal ≥ 3mm over the negative control in diameter. Historical data up to one year can be used.

14. Female subjects must be either of non-childbearing potential or if of childbearing potential use a highly effective birth control method

Exclusion criteria

Exclusion Criteria The presence of any of the following will exclude a subject from randomisation into the study:

1. Inability to comply with study procedures, required restrictions, study treatment intake or any other reason that the Investigator considers makes the subject unsuitable to participate.

2. Asthma exacerbation or chest infection requiring oral steroids and/or antibiotics, in the 3 months prior to screening or prior to randomisation.

3. History of near fatal asthma or of past hospitalisation for asthma in an Intensive Care unit.
4.Inability to perform technically acceptable spirometry measurements at screening.

5. Pregnant (or planning a pregnancy during the study) or lactating at screening or prior to randomisation.

6. Positive urine pregnancy test at screening or prior to randomisation.

7. Requires oxygen therapy, even on an occasional basis.

8. Known respiratory disorders other than asthma according to investigator's judgement. This can include but is not limited to known alpha-1 antitrypsin deficiency, active tuberculosis, lung cancer and bronchial carcinoma, bronchiectasis, sarcoidosis, lung fibrosis, pulmonary hypertension and interstitial lung disease.

9. Concomitant disease or condition that could interfere with, or for which the treatment of might interfere with the conduct of the study, or that would, in the opinion of the investigator, pose an unacceptable risk to the subject in this study.

10. An abnormal and clinically significant 12-lead ECG which may impact the safety of the subject according to investigator's judgement. N.B: Subject whose electrocardiogram (ECG) (12 lead) shows QTcF>450 males or QTcF> 470 ms for females at screening are not eligible.

11. History of hypersensitivity to β2-agonist, corticosteroids or any of the excipients contained in any of the formulations used in the trial which may raise contra-indications or impact the efficacy of the study drug according to the investigator's judgement.

12. Clinically significant laboratory abnormalities at screening indicating a significant or unstable concomitant disease which may impact the efficacy of the study drug or the safety of the subject, according to investigator's judgement.

13. Subjects with a history of chronic uncontrolled disease including, but not limited to, endocrine, active hyperthyroidism, neurological, hepatic, gastrointestinal, renal, haematological, urological, immunological, or ophthalmic diseases that the Investigator believes are clinically significant.

14. Uncontrolled cardiovascular disease: arrhythmias, angina, recent or suspected myocardial infarction, congestive heart failure, a history of unstable, or uncontrolled hypertension, or has been diagnosed with hypertension in the 3 months prior to screening or prior to randomisation.

15. History of alcohol abuse and/or substance/drug abuse within 2 years prior to screening visit.

16. Has had major surgery, (requiring general anaesthesia) in the 8 weeks prior to screening or prior to randomisation or has planned surgery through the end of the study.

17. Previous lung resection or lung reduction surgery.

18. Participation in another clinical trial and received investigational drug within 30 days (or 5 half-lives whichever is longer) prior to screening. N.B.: For biologic products with slow elimination a washout of at least 6 months needs to be met prior to screening.

19. Occupations or activities involving night shift work (i.e. subjects who do not sleep at night), jetlag or sleep disruption in the 8 weeks prior to screening or likely to do so at any time throughout the duration of the study.

Figure E1. Participant recruitment and withdrawals.

Figure E2. Randomisation of treatment sequence.

Figure E3. Chronotype distribution; the corrected Mid-sleep point (MSFsc), expressed as local time, is a proxy for "phase of entrainment" of the circadian clock.

Section E2: Baseline within-day variations

Table E1. Within-day differences in lung function and biomarkers for airway inflammation at baseline

Lung function	**4 AM**	**10 AM**	**4 PM**	**10 PM**	***p*-value***
FEV_1 (L), median (IQR) (n=25)	2.50 (1.84, 3.16)	2.83 (2.36, 3.45)	2.63 (2.22, 3.30)	2.52 (2.12, 3.18)	**<0.01**
FEV_1 percent predicted (%), median (IQR) (n=25)	75 (59-82)	86 (71-89)	82 (67, 87)	77 (65, 86)	**<0.01**
FVC (L), median (IQR) (n=25)	4.06 (3.27, 4.63)	4.27 (3.53, 4.92)	4.27 (3.33, 4.94)	4.12 (3.40, 4.72)	**0.01**
FVC percent predicted (%), median (IQR) (n=25)	91 (88, 99)	96 (89, 103)	92 (89, 102)	93 (87, 101)	**0.02**
FEV_1/FVC ratio (%), median (IQR) (n=25)	62.4 (56.8, 71.7)	70.6 (63.4, 76.8)	63.6 (57.7, 73.0)	63.8 (59.1, 71.5)	**<0.01**
FeNO (ppb), median (IQR) (n=25)	42.5 (24.0, 69.5)	49.5 (25.5, 96.5)	47.0 (27.5, 90.0)	47.0 (21.5, 84.0)	**0.01**
Blood eosinophils counts (x10^9 cells/L), median (IQR) (n=22)	0.31 (0.23, 0.51)	0.24 (0.19, 0.38)	0.25 (0.16, 0.35)	0.32 (0.21, 0.46)	**<0.01**
Serum cortisol (nmol/L), median (IQR) (n=25)	147 (75, 286)	301 (275, 347)	156 (147, 200)	71 (51, 94)	**<0.01**
Sputum % eosinophils (%), median (IQR) (n=4)	0.88 (0.19, 1.62)	-	0.75 (0.50, 1.00)	-	0.58

**Friedman test, bold: p-value <0.05*

Figure E4. Within-day difference at baseline A) FEV_1 in litres, B) FEV_1 percent predicted, C) FVC in litres, D) FVC percent predicted, E) FEV_1/FVC ration, F) FeNO (ppb), and G) blood eosinophil counts. P-value adjusted for FDR.

At baseline, the median (IQR) same-day within-individual variation in FEV_1 (calculated as maximum – minimum) was 330 (250, 420) ml and 11 (7, 13) % predicted, and 280 (200, 400) ml and 6 (4, 9) % predicted in FVC. The same-day within-individual variation in the blood eosinophil count was 0.12 (0.10, 0.17) x 10^9 cells/L and FeNO 10.5 (7.5, 16.0) ppb; two thirds (16 out of 23) of participants had the same-day variations in blood eosinophil count exceeding 0.10 x10^9 cells/L and 13 (52%) had FeNO exceeding 20% (calculated as [maximum – minimum] / mean). Only 4 participants were able to produce sputum at 4 AM and 4 PM at baseline with no differences in sputum eosinophil counts at these time points (Table 42).

Figure E5. Serum cortisol levels at different time points at baseline.

Figure E6. FEV_1 (in litres) at the end of treatment periods compared to baseline.

Figure E7. FEV_1 percent predicted (%) at the end of treatment periods compared to baseline.

Figure E8. FVC (in litres) at the end of treatment periods compared to baseline.

Figure E9. FVC percent predicted (%) at the end of treatment periods compared to baseline.

Figure E10. FEV_1/FVC ratio (%) at the end of treatment periods compared to baseline

Figure E11. The differences in the improvement in lung function at 10 PM amongst dosing regimens.

Section E3: Peak expiratory flow rate

There was an improvement of morning PEF following BD dosing compared to baseline; both BD and OD_{PM} dosing modestly improved evening PEF (Table E2, Figure E12). No difference in the diurnal variability was observed following any dosing regimen compared to the baseline (Table E2). There was no difference in the improvement of diurnal variability, morning and evening peak expiratory flow amongst all dosing regimens (Figure E12).

Table E2. The average morning and evening peak expiratory flow during the last week of run in and treatment periods in participants who completed all dosing regimens.

Dosing regimens	**Mean morning peak flow (L/min)**	**Mean evening peak flow (L/min)**	**Mean peak flow diurnal variability (%)**
Run in, median (IQR) [n=20]	387 (348, 475)	383 (340, 492)	6.0 (4.3, 8.9)

OD_{AM}, [n=20] Median (IQR), *p*-value*	404 (351, 489) *p*=0.502	401 (342, 489) *p*=0.056	5.5 (4.5,7.6) *p*=0.784
BD, [n=20] Median (IQR), *p*-value*	429 (355, 482) ***p*=0.050**	413 (346, 486) ***p*=0.038**	5.0 (3.1, 6.4) *p*=0.090
$OD_{PM,}$ [n=20] Median (IQR), *p*-value*	413 (345, 498) *p*=0.312	406 (360, 506) ***p*=0.013**	6.1 (5.2, 9.0) *p*=0.870
**Wilcoxon rank test, post treatment period compared to baseline; bold indicates statistical significance.*			

Figure E12. The changes in morning, evening and diurnal variability in peak flow during the last week of Run-in period and each treatment period.

Figure E13. FeNO (ppb) at the end of treatment periods compared to baseline.

Figure E14. Blood Eosinophils at the end of treatment periods compared to baseline.

Figure E15. Changes in blood eosinophil counts at 10 PM and 4 AM amongst dosing regimens.

Section E4: Sputum

Ten participants produced sufficient sputum at baseline, 13 following OD_{AM}, 7 OD_{PM} and 11 after BD dosing regimens. However, the number of participants who were able to produce sufficient sputum at both time points (4 AM and 4 PM) at all visits (at baseline and following each treatment regimen) were insufficient for statistical analysis (Figure E16).

Figure E16. Paired comparison in the sputum eosinophil counts (in %) at 4 AM following each treatment period vs. baseline.

Table E3. Changes in serum cortisol, blood glucose and insulin levels at the end of each treatment period compared to baseline.

Time of measurements	**OD_{AM}**	**BD**	**OD_{PM}**	***p*-value**
Changes in serum cortisol levels (nmol/L), median (IQR)				
4 AM (n=20)	-16 [-75, 28]	-69 [-184, -26]	-35 [-115, -3]	**<0.01***
10 AM (n=20)	-25 [-56, -10]	-41 [-76, -17]	-50 [-74, -2]	0.85
4 PM (n=21)	-78 [-97, -23]	-30 [-63, 17]	-6 [-45, 8]	**0.05***
10 PM (n=20)	-22 [-33, -10]	-13 [-32, 0.3]	-38 [-56, 32]	**<0.01***
Change in blood glucose levels (mmol/L), median (IQR)				
8 AM (n=21)	-0.1 [-0.1, 0.2]	-0.1 [-0.2, 0.1]	0 [-0.2, 0.1]	0.15
Change in blood insulin levels (mIU/L), median (IQR)				
8 AM (n=17)	1.6 [-1.2, 2.9]	1.4 [-0.5, 3.0]	1.1 [-0.6, 2.8]	0.75
**Pairwise comparison was carried out*				

Figure E17. Serum cortisol levels at the end of each treatment regimen compared to baseline.

Figure E18. Pairwise comparisons of serum cortisol levels amongst treatment regimens at 4 AM, 4 PM and 10 PM.

***Figure E19*.** Dynamic circadian changes in lung function parameters, FeNO, blood eosinophil counts and cortisol levels using multivariate mixed-effects cosinor modelling, adjusted for treatment sequence.

Top Panel shows cosinor analysis over time (x-axis); the dotted line indicates MESOR, vertical dotted arrows indicate Amplitude and the horizontal dotted arrows indicate Acrophase (as demonstrated in Figure 2); bottom panels demonstrates the overall mean (95% CI) of MESOR, Amplitude and Acrophase, estimated using 1000 iterations of bootstrapping. For pairwise comparison amongst three dosing regimens, p-value and FDR are presented: §FDR<0.1, *FDR<0.05, **FDR<0.01,

***FDR<0.001; ∞ *Based on 24hr period; acrophase is a measure of the timing of overall high values occurring in the cycle, expressed in negative radians.*

Figure E20. Asthma control questionnaire at baseline and following each treatment regimen.

Section E5: Adverse event and reliever mediation use

A total of 55 AEs were reported during study duration from 20 participants, of which only one was possibly related to treatment (mild dry mouth during OD_{PM} dosing). No Severe AEs were reported.

Relievers were most used in the morning and at night (Figure E21). However, there was no differences in the frequency of reliever use amongst three dosing regimens (Table E4)

Figure E21. The time-of-day when as required salbutamol were used.

Table E4. Salbutamol use during treatment periods.

Dosing regimen	**Number of participants* reported the use of rescue salbutamol during treatment period (n)**	**Frequency of salbutamol use during treatment period*, median (IQR) (n of occasions)**	**p-value[±]**
BD	15	3 (0, 19)	0.76
OD_{PM}	15	3 (0, 11)	
OD_{AM}	14	3 (0, 29)	
**Participants who completed all treatment periods (n=21); [±] Friedman test amongst 3 dosing regimens.*			

References:

1. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates R et al. Standardisation of spirometry. Eur Respir J, 2005. 26(2): p. 319-338
2. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J, 2017. 49(1).
3. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J, 2012. 40(6): p. 1324-1343.
4. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med, 2005. 171(8): p. 912-930.