

Meeting Report

Theme: Current Scientific and Regulatory Approaches for Development of Orally Inhaled and Nasal Drug Products

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Pharmacodynamic Studies to Demonstrate Bioequivalence of Oral Inhalation Products

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Abstract. In the session on “Pharmacodynamic studies to demonstrate efficacy and safety”, presentations were made on methods of evaluating airway deposition of inhaled corticosteroids and bronchodilators, and systemic exposure indirectly using pharmacodynamic study designs. For inhaled corticosteroids, limitations of measuring exhaled nitric oxide and airway responsiveness to adenosine for anti-inflammatory effects were identified, whilst measurement of 18-h area under the cortisol concentration–time curve was recommended for determining equivalent systemic exposure. For bronchodilators, methacholine challenge was recommended as the most sensitive method of determining the relative amount of β -agonist or anti-muscarinic agent delivered to the airways. Whilst some agencies, such as the Food and Drug Administration (FDA), do not require measuring systemic effects when pharmacokinetic measurements are feasible, the European Medicines Agency requires measurement of heart rate and serum potassium, and some require serial electrocardiograms when bioequivalence is not established by pharmacokinetic (PK) studies. The Panel Discussion focused on whether PK would be the most sensitive marker of bioequivalence. Furthermore, there was much discussion about the FDA draft guidance for generic fluticasone propionate/salmeterol. The opinion was expressed that the study design is not capable of detecting a non-equivalent product and would require an unfeasibly large sample size.

KEY WORDS: inhaled bronchodilators; inhaled corticosteroids; methacholine; pharmacodynamics.

INTRODUCTION

This paper is part of a series of reports from the “Orlando Inhalation Conference—Approaches in International Regulation” co-organised by the University of Florida and the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) held in March 2014.

Regulatory agencies differ between countries as to what is required for approval of a new product as a generic or therapeutic equivalent. For example, the US Food and Drug Administration takes a “weight of evidence approach”. They require three types of studies: an *in vitro* equivalence study, a pharmacokinetic study to demonstrate equivalent therapeutic

equivalence and a pharmacodynamic or clinical study to demonstrate bioequivalence. Canada requires *in vitro* studies and a pharmacokinetic study. If bioequivalence is not demonstrated, then they require a pharmacodynamic study. On the other hand, in the European Union, a therapeutic equivalent product (designated as a “hybrid product”) potentially can be in principle approved with an *in vitro* study (see article on global regulatory considerations in this issue).

If a pharmacodynamic study is required, the study design will differ for inhaled corticosteroids and bronchodilators. We summarise here the papers presented in the section of the program listed as “PD to Demonstrate Efficacy and Safety”.

INHALED CORTICOSTEROIDS

Topical Efficacy

The focus of Dr. Daley-Yates’ presentation was on the need to have validated biomarkers to assess the delivery of inhaled drugs to the site of action in the airways (1). Biomarkers should be relevant to the disease process both biologically and temporally. Clinical outcomes based on lung function, symptoms and systemic drug concentrations are downstream of the site of action and do not necessarily reflect

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the rate and extent of drug availability at the site of action in the airways.

For inhaled corticosteroids (ICS), there are a large number of potential biomarkers that could be used. Some are likely to be more useful than others based on their temporal association with drug at the site of action (Fig. 1). Although symptoms and lung function are clinically relevant outcomes, they are far downstream of the drug site of action and have many sources of variability beyond drug delivery, whereas early anti-inflammatory events have the potential to be more direct measures of drug delivery. These include sputum eosinophil counts and rapidly released mediators such as exhaled nitric oxide (eNO).

Exhaled nitric oxide has been well studied. Everyone has eNO in their breath, but the concentration is higher in patients with asthma where its levels are thought to correlate with active inflammation and eosinophil numbers (2). It is inhibited by ICS therapy, taking about 2 weeks to get the maximum effect, about a 60% reduction, with an ED_{50} of approximately 100 mcg/day of budesonide or beclomethasone dipropionate (Daley-Yates, unpublished). Therefore, measuring the ability of an ICS to reduce elevated eNO may have utility as a marker of drug delivery to the site of action in the airways.

Challenge models measure airway responsiveness and ICS protection of that. Some directly act on smooth muscle (methacholine and histamine), others indirectly *via* activated immune cells and cause release of inflammatory mediators. Although further downstream than eNO release, challenge models have greater biological relevance since they evoke a cascade of anti-inflammatory events similar to asthma (Fig. 1). Indirect challenges such as adenosine-5'-monophosphate (AMP) are potentially the most relevant candidates, as the cascade of events is closer to the asthmatic response. AMP acts indirectly by stimulating cells that participate in the immune response to release mediators that cause airway narrowing. This is in contrast to widely used challenge agents like methacholine that act directly on airway smooth muscle to cause bronchoconstriction. Measuring the ability of an ICS to block the response to AMP may therefore have utility as marker of drug delivery to the site of action in the airways.

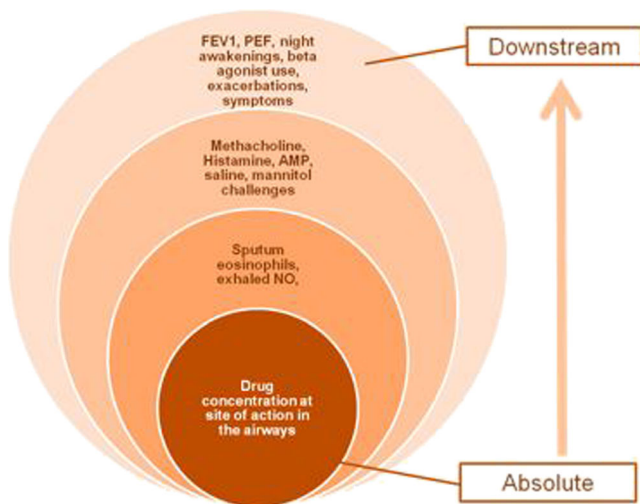


Fig. 1. Relationship between drug at site of action and biomarkers of anti-inflammatory effects in the airways

Dr. Daley-Yates explored the biomarker potential of AMP challenge and eNO further by describing a cross-over study in 49 mild asthmatics (3) that investigated five dose levels of fluticasone propionate (FP) and placebo, dosed for 5 days bid. Subjects were screened for AMP responsiveness and lack of variability. Baseline eNO was 64 ppb. There was a minimum washout period of 14 days. Exhaled NO was measured at baseline and on day 5. AMP challenge was performed 2, 14 or 26 h post-dose on day 5 in each period in each subject. All doses reduced eNO compared to placebo, but most of the effect was seen at the lowest dose (Fig. 2). The data were well described by an E_{max} model with ED_{50} of 18.4 mcg/bid; however, this was not well estimated since the ED_{50} was smaller than the lowest dose of 50 mcg BID.

For AMP, all doses were significantly different from placebo at the 2-h post-dose challenge time point (Fig. 3), and the E_{max} was 31 mcg/bid. Although this was higher than the eNO ED_{50} , it was still less than the lowest FP dose. For the 14- and 26-h post-dose challenge time points, only the 100-mg bid and 500-mcg bid doses were significantly different from placebo (Fig. 3). Both time points showed a reduced E_{max} effect but a higher ED_{50} compared to the 2-h time point. The interpretation of this was that the protective effect of the steroid had worn-off at 26 h for lower doses, but it also appeared that the earlier challenge or challenges at 2 and 14 h may have attenuated the effect of the later challenges. Other studies have investigated this aspect (4). Taken together, these studies indicate that a single challenge at 14 h post-dose would likely be optimal to assess a dose response.

In addition, Dr. Daley-Yates described some further exploratory work looking at the feasibility of using AMP and eNO for bioequivalence (BE) testing in the way it was done in this study. It was estimated that standard BE criteria of T/R ratio within $\pm 20\%$ on the dose scale would equate to $\pm 8\%$ or $\pm 3\%$ on the AMP PC_{20} or eNO ppb scale, respectively. For standard BE 80% power and 0.05 alpha, 1946 and 844 subjects, respectively, would be needed in a

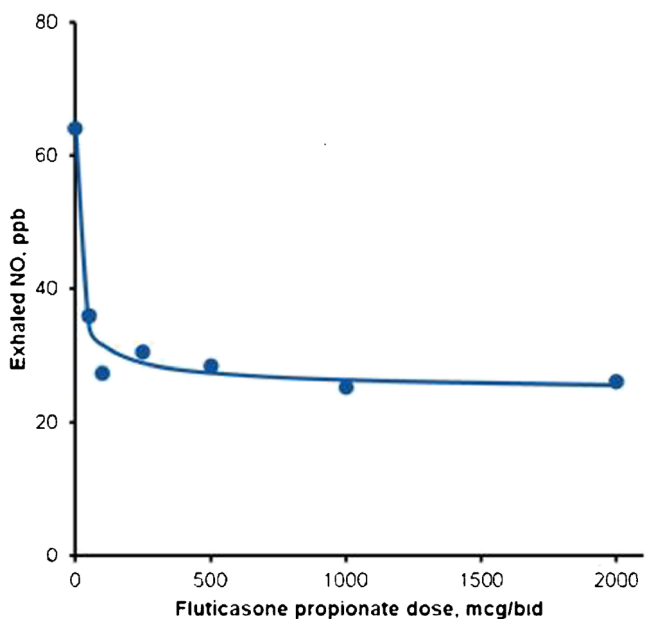


Fig. 2. Dose response for fluticasone propionate doses on exhaled nitric oxide (adapted from 1)

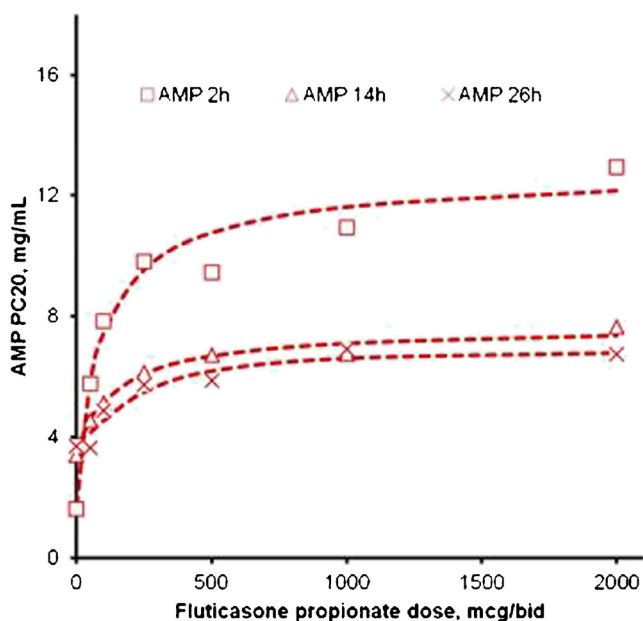


Fig. 3. Dose response for fluticasone propionate doses on exhaled nitric oxide (adapted from 1). Zero dose is placebo

cross-over design. This was regarded as a worst-case scenario as the protocol was not optimised. Therefore, this aspect was explored in more detail for the eNO data using the relative potency approach and non-linear mixed effects modelling (5) similar to that described in the presentation by Dr. Kandala (6). The model gave a slightly higher estimate for ED_{50} of 24 mcg/bid. The model was used to simulate the outcome of various trial designs, 300 simulations in each case (5). Using a design with three dose levels of reference and one of test (100 mcg) and 64 subjects, only half the trials had point estimates within 80–125% BE limits and a large subject numbers would be needed for BE testing. Even using the best-case scenario with five dose levels for T and R , and placebo with complete cross-over, including a dose around ED_{50} at 25 mcg, large subject numbers were still needed for 80–125% BE testing when T and R are assumed to be the same (relative potency=1).

Dr. Daley-Yates concluded that exhaled nitric oxide and adenosine-5'-monophosphate challenge both have potential as markers of drug availability at the site of action in the airways. They have the key attributes of biological relevance, rapid responses and short washout periods, and they are relatively easy to measure. However, it was more difficult to make general conclusions about the potential for biomarkers in BE testing for inhaled anti-inflammatory drugs when the reality is that the area has not been fully explored experimentally. For example, study designs for eNO should be evaluated using the area under the effect curve rather than single time points and also exploring the on- and off-set of effect, shorter dosing, and/or once daily dosing regimens. The AMP challenge dose–response could be optimised by investigating the duration of the dosing period and timing of challenge post-dose. All of these may improve the sensitivity to detect a dose response in the clinical dose range.

Dr. Kandala presented the results of Monte Carlo Simulations from a statistical model to determine if it is

feasible to use cross-over studies for assessing pulmonary BE of inhaled corticosteroids using eNO as the outcome (6). In various scenarios, he varied the baseline eNO, maximum effect (E_{max}) and the dose of ICS producing 50% of the E_{max} (ED_{50}). Two hundred datasets were simulated for each scenario, and 1000 bootstrap datasets were generated to obtain the 90% confidence interval for relative bioavailability (metric used for BE assessment) of the test product with respect to the reference product. Power was defined as the percentage of 200 simulated datasets that passed the BE criteria (90% confidence interval of relative bioavailability within limits of 0.67–1.5). He confirmed Dr. Daley-Yates' observation that the highest power is achieved when the test dose is close to the ED_{50} . In addition, he found that the eNO method was only feasible with a cross-over design when subjects with a high-baseline eNO are selected (>90 ppb) and the BE criteria is relaxed to 0.67, 1.5. The problem with this finding, however, is that it is difficult to find volunteers with such a high eNO. In another pilot study to evaluate eNO, Weiler *et al.* (7) screened 105 subjects to find 22 subjects with an $eNO \geq 60$ ppb and only 17 completed the study.

Systemic Safety

Dr. Hermann discussed the design and conduct of hypothalamic–pituitary–adrenal (HPA) axis studies comparing the systemic safety of orally inhaled products (OIPs) containing glucocorticosteroids (ICS) (8).

Measures of ICS-mediated adrenal suppression (*i.e.* inhibition of cortisol secretion) are generally accepted as most sensitive and accessible markers for adverse systemic ICS effects. Among various approaches, quantification of non-stimulated circadian cortisol secretion [*i.e.* 24-h serum/plasma cortisol area under curve (AUC)] represents the most sensitive marker for the assessment of systemic ICS exposure and potency. Accordingly, the approach is recommended by the European Medicines Agency (EMA) as the most preferred safety pharmacodynamic (PD) study methodology for the examination of systemic effects of ICS-containing OIPs (9). However, the EMA OIP Guideline contains a couple of misconceptions and provides little useful methodological advice (Table I). In contrast, for the ICS component of a generic for the combination of fluticasone/salmeterol (Advair), the Food and Drug Administration (FDA) does not require HPA-axis studies (10). They would only require such studies if pharmacokinetics (PK) cannot be performed (Dr. Sau Lee, Office of Generic Drugs, 10/14/14, personal communication).

In addition, Dr. Hermann presented data of a systematic quality review of published HPA-axis papers ($N=80$ original manuscripts, published between January 1, 2005 to June 30, 2012; unpublished data on file), which indicate widespread failure of respiratory study groups to translate existing endocrinological knowledge on confounding intrinsic and extrinsic factors (see Table II) and state-of-the art methodology to HPA-axis studies of ICS-containing OIPs. It was said that for second-entry ICS-containing OIP products aiming to demonstrate comparable systemic safety (*i.e.* safety PD equivalence) *versus* a reference listed product by quantification of circadian cortisol secretion confounding intrinsic and extrinsic factors (Table II) need to be more carefully

Table I. EMA Recommendations for HPA-Axis Studies (9)

EMA guideline statements	Comments
Organ system/biomarker: PD assessment of systemic effects of ICS in adults is to assess the effect on the HPA axis Primary PD read-outs: Repeated assessment of the change from baseline in 24-h plasma cortisol as measured by AUC (as the primary variable) and C_{max} Dose levels to be examined: Inhalation of the maximum recommended total daily dose regimen of the ICS, together with the assessment of a lower dose regimen	Requirement of HPA-axis assessment and recommendation of 24-h plasma cortisol AUC values as the primary outcome variable in-line with established evidence Requirement of equivalent cortisol C_{max} values as additional outcome variable, does not acknowledge the known pulsatile ACTH/cortisol release characteristics and, hence, appears inappropriate (<i>i.e.</i> PK-minded approach to PD-outcomes!) Requirement of highest recommended dose-level inconsistent with requirements of “assay sensitivity” (<i>i.e.</i> to assessing PD effects at the steep part of the dose response curve) → HPA-axis studies at the highest recommended dose-level of ICS are unlikely to be discriminative for product performance characteristics! Lack of robust evidence whether or not steady-state studies may be more discriminative as compared to single-dose studies
Duration of treatment: Must be justified and must ensure that PK steady-state has been reached Population: Study should be carried out in patients with asthma	Requirement of asthma patients as study population carries many methodological draw-backs, <i>e.g.</i> • lower pulmonary deposition • inter-occasion variability of airway caliber • already compromised HPA-function • higher probability of GCR-resistance • practical and ethical constraints with wash-out of concomitant asthma medications, in particular in those patients assigned to low-dose treatments groups → asthma patients represent a highly variable, poorly accessible and hardly sensitive study population; risk–benefit and methodological/ethical constraints appears overall unfavourable Rigorous control of stability of circadian conditions and lifestyle should be observed throughout the entire study and not just at the days of HPA assessment
Setting: Controlled, fully tested environment (<i>i.e.</i> patients should be studied as in-patients on those days when assessments are being carried out)	

ICS inhaled corticosteroid(s)

addressed and rigorously controlled to accurately quantify ICS-mediated HPA-axis effects, thereby managing the risk of non-product-related study failures. Accordingly, the presentation reviewed and summarised methodological key quality criteria of HPA-axis studies aiming for accurate quantification of systemic HPA-axis effects of ICS products.

It was emphasised that a detailed knowledge of the physiology and pathophysiology in the regulation of cortisol secretion is of utmost importance for the proper design and conduct of HPA-axis studies (Table II).

For the accurate and reproducible assessment of 24-h cortisol secretion (*i.e.* serum/plasma cortisol AUC), the pulsatile cortisol secretion pattern represents a major challenge as incidental capturing of cortisol pulses may significantly distort the respective AUC outcome (Fig. 4). In this context, it is important to note that the cortisol pulse frequency is significantly higher in men compared with women (18 vs.10 pulses/24 h). Furthermore, it needs to be considered that the overall probability to incidentally capture cortisol pulses during 24-h profiling is also schedule

Table II. Intrinsic and Extrinsic Factors Altering HPA-Axis Function

Intrinsic factors	Extrinsic factors
Endocrinological diseases (Cushing’s)	Stress conditions, <i>e.g.</i> job-related (pre)burn out, private distress and conflicts, school test taking, <i>etc.</i>
GCR resistance due to genetic GCR-variants/polymorphisms	Abrupt changes in circadian rhythm such as shift-working or excessive weekend parties, <i>etc.</i>
Psychiatric disorders, <i>i.e.</i> depression	Excessive alcohol consumption
Sleep disorders, <i>i.e.</i> insomnia, sleep-disordered breathing (SDB)	Factors altering GCR-responsiveness/sensitivity, <i>e.g.</i> smoking (<i>i.e.</i> acquired GCR resistance)
Chronic liver diseases, <i>e.g.</i> cirrhosis	Dietary changes, in particular carbohydrate (<i>via</i> blood glucose–cortisol axis) and potassium-rich diets
Obesity	Seasonal changes (daylight times)

GCR Glucocorticoid receptor

dependent, *i.e.* increases with increased sample frequency. It was suggested that specific blood sampling and data evaluation strategies are required to mitigate the impact of incidental capturing of cortisol pulses. For example, it was recommended to obtain, *e.g.* three samples at each collection time point (about 10 min apart), and to use the median of these three samples for each individual time point. Regarding the primary read-out of cortisol secretion, it was proposed to consider average cortisol concentrations (C_{av}) in addition to or instead of AUC values, as these might be less sampling-schedule dependent (*i.e.* fraction of distorted AUC area is schedule dependent) and more robust (*i.e.* less sensitive) against single data-point outliers (Fig. 4).

Dr. Hermann further emphasised that little attention has been paid in historical studies on the existence of more or less sensitive time-periods of the 24-h cortisol profile, when it is aimed for the assessment of ICS-mediated feedback suppression. Available data indicate that the quiescent period (*e.g.* from 8:00PM to 2:00AM) hardly provides sensitive or useful information on ICS-mediated cortisol suppression. It was therefore recommended to focus blood sampling on the extended acrophase (*i.e.* from 2:00AM to 8:00PM) as most sensitive time period of cortisol profiles for detection of ICS-mediated suppression, *i.e.* to capturing actually 18-h cortisol profiles instead of 24-h profiles.

Dr. Hermann suggested implementing the following assessments as standard examinations for the selection of a well-defined HPA-axis study population.

Include only healthy subjects. Inquire about stability of diurnal rhythm/lifestyle and regular sleep habits, *i.e.* exclude shift working, travels across time zones (jet lag) and excessive weekend parties; exclude subjects with current presence of stressful conditions, *e.g.* job-related (pre)burn out, private distress, exams, *etc.*; exclude subjects with current presence of sleep disturbances/insomnia; exclude subjects with excessive alcohol consumption and/or significant liver disease; exclude subjects with current evidence of depression disorders, *e.g.* apply, Well Being Five' Questionnaire (11); check for glucocorticoid receptor (GCR) sensitivity, *e.g.* by low-dose dexamethasone suppression test (12); screen all subjects for physiological (*i.e.* "normal") and reproducible cortisol profiles (*i.e.* 2 replicate 24-h cortisol screening assessments 3 days apart), including formal profile analysis *prior* to enrolment.

GCR resistance or insensitivity describes the inter-subject variability in sensitivity to ICS feedback suppression. The condition can be either inherited (*i.e.* genetic variants/polymorphisms) or acquired (*e.g.* depression, asthma, alcohol, smoking, *etc.*; see Table II). Variation in GCR sensitivity also exists within the normal population with mechanistically unexplained sources [such as non-suppression in dexamethasone suppression test in 4 to >9% of subjects across studies (12)]. Most patients with GCR resistance display increased plasma ACTH and serum cortisol concentrations and elevated urinary cortisol secretion, whilst the diurnal rhythm is maintained (13). Such subjects should be excluded.

Published evidence indicates high reproducibility of 24-h cortisol profiles in healthy adult subjects synchronised for

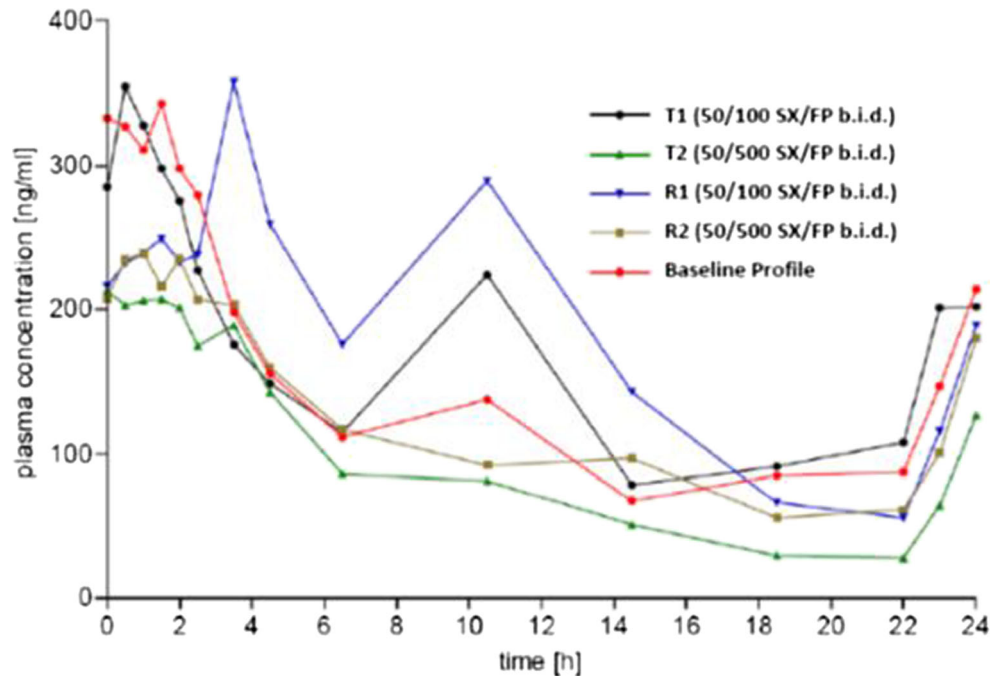


Fig. 4. Serum cortisol-time curves of an individual healthy young, white female subject participating in a single-centre, randomised, double-blind, double-dummy, four-period, 4-day repeat-dose crossover study with baseline evaluation comparing two strengths (*i.e.* 50/100 and 50/500 μg) of a salmeterol/fluticasone propionate containing TEST DPI (T1 FP low dose; T2 FP high dose) and Seretide Diskus® (R1 FP low dose; R2 FP high dose). There are two irregular profiles (*blue and black curves*) with high cortisol levels *versus* the BL-profile despite (low dose) FP treatment and atypical daytime peaks. Findings may point either to sleep shifts or stress exposure or capturing of cortisol pulses in two out of four individual profiles. Note the substantial impact on AUC outcomes, depending on sampling schedule

their diurnal activity and nocturnal rest (14). However, historically, only few HPA-axis studies in the respiratory field were conducted with subjects under controlled diurnal and lifestyle conditions (*i.e.* with complete in-house confinement). For a “thorough” HPA-axis study comparing ICS-mediated HPA-axis suppressive effects, it is indispensable to confine subjects in-house for the entire study duration and to synchronise them for their diurnal activity and nocturnal rest. Subjects should also be non-smokers, refrain from alcohol throughout the study, and should receive standardised meals. Whilst ideal, confining subjects to a clinical research unit for an entire study period is not very practical and will make it difficult to recruit volunteers.

Cortisol concentrations are measured by high-performance liquid chromatography (HPLC), HPLC/MS/MS or ELISA assays which are the easiest to perform.

INHALED BRONCHODILATORS

Bronchoprovocation with Methacholine

There were two presentations on the use of methacholine bronchoprovocation as a bioassay for the relative amount of beta agonist delivered to the airways. Dr. Hendeles provided evidence that the dose–response curve, using FEV₁ alone, is relatively flat and not capable of distinguishing between two adjacent doses of the same beta agonist delivered by the same device (15). Therefore, it would not be possible to detect a generic or hybrid product that would deliver 50% less or 200% more drug than the reference product. Previous studies have indicated that bioassay with methacholine is able to distinguish between different beta agonists (16), the same drug delivered by two different devices (17) and two MDIs with different propellants using the same drug (18). Dr. Hendeles presented data demonstrating that, for the long-acting beta-agonist formoterol, a study in ten adults with mild asthma found that both the 12 and the 24 µg doses, delivered by Aerolizer, both produced a similar 14% mean increase in FEV₁ after 1 h (19) (Fig. 5). In contrast, the provocative concentration of methacholine causing a 20% decrease in FEV₁ (*i.e.* PC₂₀FEV₁) was 7 mg/mL after the 12 µg and 16 mg/mL after the 24 µg, a 2.4-fold difference ($p < 0.001$) (Fig. 6). Furthermore, he

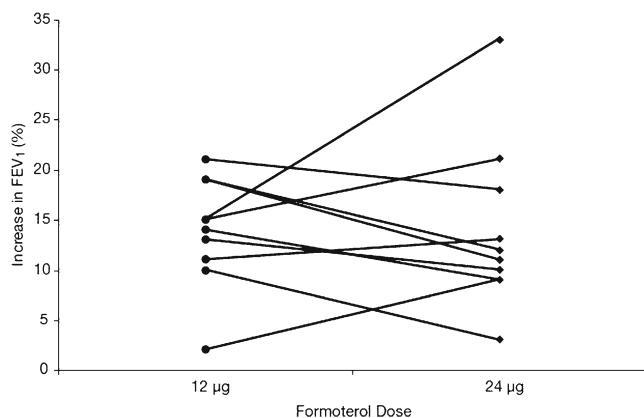


Fig. 5. Bronchodilator response measured as percent increase in forced expiratory volume in 1 s (FEV₁) 1 h after administration of single doses of formoterol 12 and 24 µg (adapted from 19)

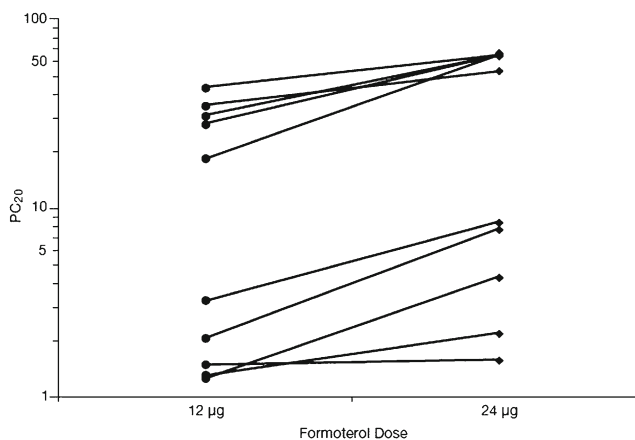


Fig. 6. Provocative concentration of methacholine required to decrease the forced expiratory volume in 1 s by 20% (PC₂₀) measured 1 h after administration of single doses of formoterol 12 and 24 µg, on separate days, at the same time of day, in ten patients who completed the study. A significant dose–response relationship was noted; the geometric mean (95% confidence interval) was 7 mg/ml (2–22 mg/ml) after the 12-µg dose and 16 mg/ml (5–45 mg/ml) after the 24-µg dose ($p < 0.001$) (adapted from 19)

presented data on the delivery of formoterol by Aerolizer and another dry powder inhaler available in Europe, the Novolizer (20). There was a relative potency of 1.13 for Novolizer/Aerolizer with a 90% confidence interval of 0.94 and 1.38 in the 43 subjects who completed this two-centre study. Using Monte Carlo simulations, Dr. Kandala found similar results for the same study with an estimated ED₅₀=7.8 mcg, also below the lowest dose tested (6). As pointed out in his presentation, relative bioavailability could be determined using the dose-scale approach.

Dr. Hendeles’ presentation concluded with a discussion of the statistical power of the methacholine bioassay. Available evidence indicates that the ratio of within subject variability (s) and slope of the dose–response curve (b) relate to the statistical power of the methacholine bioassay. The smaller the s/b ratio, the greater the power (17). Therefore, in using this methodology, it is important to strive to minimise variability and maximise dose–response slope. The variability can be decreased by improving the delivery method, having a central pharmacy prepare the dilutions of the methacholine, paying assiduous attention to performance of the FEV₁ and selecting subjects who are less likely to have day to day variability. As Dr. Darken of Pearl Therapeutics pointed out in his presentation, the reference product may have variability that will affect the sample size calculation (21).

In contrast to Dr. Hendeles’ conclusion, Mr. Scott Haughie of Mylan concluded the opposite (22). His group conducted a randomised, crossover study comparing placebo and two doses of albuterol and two doses of salmeterol. They found a 1.29-fold increase in PC₂₀ between 90 and 180 µg of albuterol and a 1.34-fold increase in PC₂₀ after salmeterol 100 µg compared to salmeterol 50 µg delivered by dry powder inhaler. The results for albuterol, in particular, were inconsistent with several previous studies, probably because they began the methacholine challenge 1 h after administration of albuterol, whereas in the other studies, the challenge was begun 15 min after administration. Ahrens *et al.* (16) demonstrated that there is a highly linear relationship

between log of PC₂₀ and time after inhaler administration for albuterol. Two hours after administration, the PC₂₀ is less than half as much as the PC₂₀ measured at 30 min. Thus, the small difference that was observed in the Mylan study had to be related to the delay in starting the methacholine challenge. Since it takes 45 min to 1 h to perform a challenge after albuterol, it is likely that they reached the PC₂₀ at approximately 2 h after administration.

In the Mylan study, the difference they found for salmeterol was consistent with a previous study by Palmqvist *et al.* (23), but inconsistent with studies by Derom *et al.* (24) and Higham *et al.* (25). One explanation for these differences might relate to how much beta agonist was used prior to beginning the study. For example, because of the potential for tachyphylaxis, subjects who use LABAs prior to entering the study or those who frequently use short-acting beta agonists may have a blunted response to methacholine.

The FDA draft guidance for generic albuterol products offers a choice between a bronchodilator or bronchoprovocation with methacholine (26). However, the available data suggest that most bronchodilator studies will not demonstrate the assay sensitivity specified in the guidance. Since anticholinergics are specific inhibitors of methacholine, a bioassay with methacholine should be useful for ipratropium and tiotropium as well.

Dr. Horhota (27) presented a paper on the administration of the combination of tiotropium and salmeterol from a second-generation HandiHaler compared to the combination administered as their marketed single entity products. *In vitro*, PK and pharmacodynamic endpoints of the combinations and the reference products were compared in an open-label four-way cross-over study in 50 chronic obstructive pulmonary disease (COPD) patients at steady state. The details of this study are presented elsewhere in this series as a separate standalone paper. Briefly, he found that the *in vitro* inhalable fractions for the new combination product met current criteria for *in vitro* equivalence. However, PK bioequivalence was surprisingly not achieved for both tiotropium and salmeterol with the new product. Plasma concentrations were higher for the new combination compared to the singly administered products. Higher plasma concentrations of salmeterol were also reflected in electrocardiographic (QTc) changes that correlated directly with C_{max} and T_{max}. Not unexpectedly, there was no difference in

pulmonary function profiles between both combination administrations, although there was clear differentiation in FEV₁ and FVC curves for the combinations *versus* their single entity references. Dr. Horhota concluded that completely dispensing with pharmacodynamic assessments when comparing the efficacy and safety of test and reference products is not supported by current scientific evidence. He further argued that appropriately powered crossover studies in patients with parallel PK and PD measurements represent a suitable risk management tool for the assessment of product equivalence in the case of beta agonists and anticholinergics (see full report in this issue).

Systemic Safety

The requisite PD data to establish the safety of generic β₂-agonist formulations differ between the EMA (9) and Health Canada (28) (Table III), whilst the FDA does not require a PD safety study if PK can be measured (10, 26).

This inconsistency across regulatory authorities is perhaps surprising, given that simple clinical models to evaluate the comparative systemic safety of β-agonists are long established (29, 30) and have been shown to be reproducible (31, 32). Furthermore, a fundamental principle of any study intended to evaluate equivalence, whether of safety or efficacy, between two products is that it should be sensitive to detect differences in potency or dose delivery should they exist; *i.e.* the study must have assay sensitivity. To fulfil this requirement, an equivalence study must include at least two dose levels of test and/or reference products (33, 34).

The model described by Bennett and Tattersfield in 1997 (29) and subsequently elaborated by Guhan *et al.* (30) satisfies these requirements. Briefly, the study comprises:

- The administration of single doses
- At least one supratherapeutic dose level. If a therapeutic dose level is also included it should be the highest approved dose level (to limit the likelihood of it being a no-effect dose in the PD model)
- Two- or threefold dose level increments (higher dose level multiples may be required in efficacy PD studies)
- Cardiovascular (heart rate, systolic and diastolic blood pressure and QTc interval) and biochemical (plasma potassium and glucose) endpoints, which

Table III. Summary of EMA and Health Canada PD Safety Study Requirements

	EMA (9)	Health Canada (28)
Dose levels	Maximum dose level	Therapeutic and supratherapeutic dose levels
Endpoints	QTc, serum K ⁺ , plasma glucose	Heart rate, tremor, serum K ⁺
Single or multiple dose	Not stated—efficacy PD requirements imply single-dose study acceptable for safety PD	Single-dose study
Statistical analysis	Not stated—efficacy PD requirements and single-dose level design imply effect scale analysis acceptable	Not stated—efficacy study requirements imply relative potency approach acceptable
Comment	Inconsistency in dose level requirements for ICS <i>versus</i> β-agonist PD safety studies: for ICS guideline stipulates 2 dose levels (maximum and lower dose level); for β-agonist guideline stipulates 1 dose level only (maximum) 2 dose levels in β-agonist safety PD would be in keeping with key principle of assay sensitivity advocated by guideline	No standardised scales to assess tremor

exhibit steep dose–response. Note that the relative effect of different β -agonist molecules may vary dependent upon the endpoint employed (30), and this should be considered when defining a primary endpoint.

Both relative potency (dose scale) (29, 30) and effect scale (31, 32) approaches have been used to assess such studies, and both would appear to be feasible methods in view of the steepness of the dose–response slopes that are typically observed. Although there does not appear to be consensus as to the appropriate non-inferiority margins with either approach, the inclusion of several dose levels within the model allows for a within-study examination as to whether any pre-specified margin is appropriate. The examination of food effect upon PD outcomes within the same study (as performed by Guhan *et al.*) (30) is also useful as it allows the magnitude of β -agonist-related systemic effects that are observed to be qualified and provides additional context with which to discuss the study results.

NOVEL BIOMARKERS

Functional Respiratory Imaging

Dr. De Backer presented a study on the addition of roflumilast (Daxas®, Takeda and Daliresp®, Forrest), a selective phosphodiesterase type 4 inhibitor to bronchodilator/ICS combination treatment in COPD patients (35) using functional respiratory imaging (FRI) (36). Inhalation therapy in COPD patients is often a combination of long acting beta 2 agonist (LABA), long acting anti-muscarinic agents (LAMA) and ICS. One of the main challenges in developing novel anti-inflammatory compounds such as roflumilast is that the current gold standard endpoints such as the forced expiratory volume in 1 s (FEV_1) lack the sensitivity to detect subtle differences in the respiratory system. FRI allows detecting changes in the lungs and airways with higher accuracy and hence could be used to describe the effect of anti-inflammatory interventions. The study he presented aimed to assess the mode of action of roflumilast as add-on to LABA/LAMA/ICS triple therapy in severe COPD patients. In addition, the study aimed to identify the characteristics of the responders.

Methods

Forty-one patients were randomised to receive roflumilast or placebo. At baseline and after 6 months of treatment pulmonary function tests, exercise tolerance tests and FRI were performed and patient reported outcomes were measured.

Findings

A significant improvement in FEV_1 of 66 ± 120 ml ($p=0.01$) was observed in the roflumilast group compared to baseline. The response was driven by a subset of responders ($n=8$) with a change in FEV_1 exceeding the measurement error of FEV_1 recently determined to be 120 ml (37). The responders experienced greater dynamic

hyperinflation during exercise at baseline compared to the non-responders as determined by the Borg fatigue score. FRI parameters indicated regional changes in hyperinflation after treatment with roflumilast leading to an improvement in PFT, patient reported outcomes and exercise tolerance (Fig. 7).

Interpretation

The anti-inflammatory characteristics of roflumilast seem to reduce inflammation in the smaller airways leading to a reduction in hyperinflation. The reduction in hyperinflation appears to be associated with an improved ventilation of

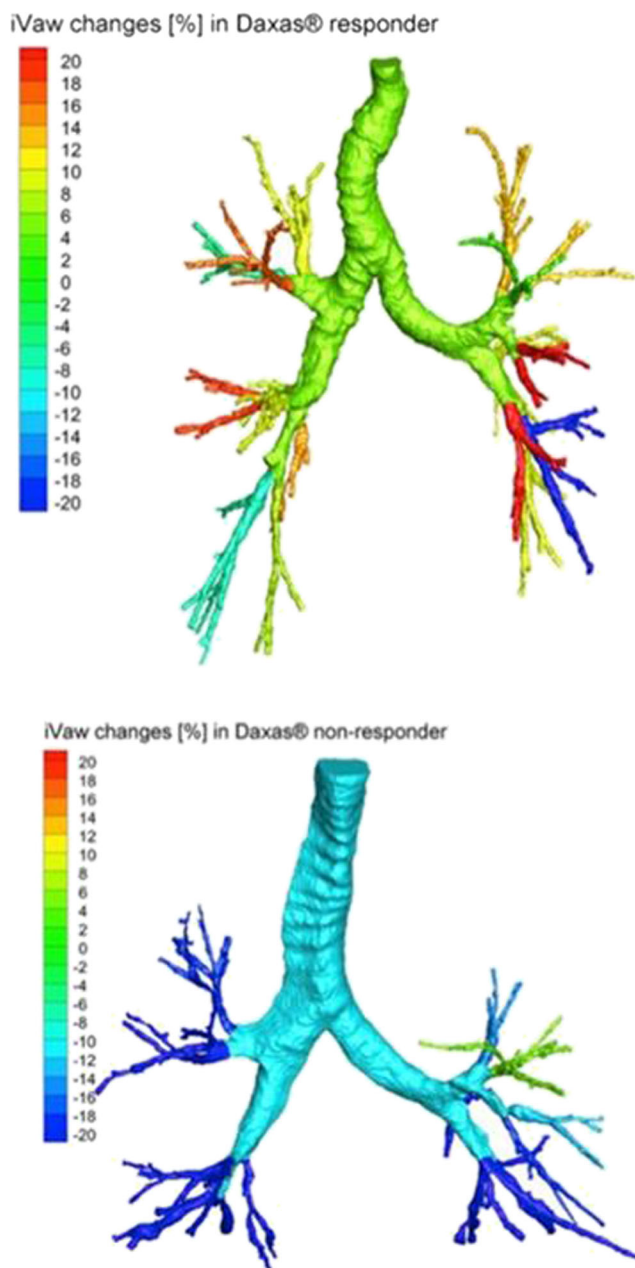


Fig. 7. Changes in iVaw in a patient with a large response ($FEV_1 > 5\%$) to the Roflumilast treatment (top). Changes in iVaw in a patient with no response ($FEV_1 \leq 5\%$) to the Roflumilast treatment (bottom)

these areas; hence, more air is going to the lobes that experience a reduction in air trapping. Consequently, the internal airflow distribution (IAD) changes in the responding patients. The change in this flow enhances the deposition of the LABA/LAMA/ICS therapy. Areas that are better ventilated also receive more inhaled particles, since inhaled particles tend to follow the internal airflow. Since drug particles are now reaching other, previously undertreated areas, clinical improvements can be observed in terms of improved FEV₁, improved 6 min walking distance and significantly more FRI based bronchodilation. Patients who suffer from dynamic hyperinflation at baseline tend to benefit most from roflumilast. Most likely, these patients have the largest amount of sub-optimally treated regions in the lungs.

The study results suggest that, in people who are prone to dynamic hyperinflation during exercise, roflumilast causes a reduction in regional hyperinflation, a redistribution of air and ICS/LABA/LAMA particles, the latter causing an improvement in FEV₁, exercise tolerance, *etc.* This improvement is not seen in the non-responders. Apparently in this group, roflumilast does not reduce the hyperinflation leading to a redistribution of the ICS/LABA/LAMA. We could, therefore, hypothesise that, in this non-responding cohort, the chronically undertreated areas remain undertreated leading to a further increase in regional hyperinflation and a reduction in airway volumes. This would be an interesting topic for further research, but having an indication for a responder phenotype is already a major step forward in the process of positioning this drug.

The findings of this study are relevant for two main reasons. Firstly, the current study is the first study to report the effect of a PDE4 inhibitor in addition to ICS/LABA/LAMA triple therapy. Secondly, more sensitive, image-based endpoints provide additional insights into the mode of action of anti-inflammatory compounds and provide a basis for responder phenotyping. The latter will be important when considering the development of novel, often expensive anti-inflammatory compounds for respiratory diseases. The current study provides hypotheses that need to be confirmed in larger clinical trials.

PANEL DISCUSSION

Audience: Why measure cortisol suppression? Why not measure exposure directly since cortisol suppression is directly linked to PK anyway?

Panel: It can be done. PK bioequivalence boundaries can be widened instead of doing PD study. This project is currently being worked on in Dr. Hochhaus' lab.

Audience: Could the Panel comment on the FDA guidance for bioequivalence when the biomarker itself is not sensitive.

Panel: This has to be seen in the context of the weight of evidence approach. It is recognised (by the FDA) that a clinical endpoint study is much less sensitive than any other

study designed in BE testing. It is done to confirm that you are getting the same effect with the reference product that you would with the test. FDA does not believe that it is the most sensitive method to detect formulation differences.

Panel: The Brazil Regulatory authority viewpoint—PK studies need to be shown in order to show BE. There are too many challenges at the moment to consider PD biomarkers.

Audience: What if you get perfect PK BE and then do a PD study with three doses? Two out of three of those doses pass. Do all three have to meet PD BE criteria or would you approve two out of three?

Panel: It is possible that the two that passed will be approved (by FDA).

Audience: Can the Advair guidance from the FDA be used for other inhalers?

Panel: FDA will provide more product specific guidances and use the common points that apply to the inhalers from the Advair guidance. They will focus on what is unique to each inhaler.

Audience: With regards to the Advair guidance, if I have a generic device that is identical to the Diskus, for example, will a comparative human factor study be considered?

Panel: This will have to be discussed further (by the FDA).

Audience: There is the critical path initiative to find new biomarkers. Are there any other ways that we can find new biomarkers?

Panel: Companies can discuss positive biomarkers with the FDA during the development process so that the focus is not just BE but also finding new biomarkers.

Conflicts of Interest Dr. Hendeles is the PI on research grants to the University of Florida for GlaxoSmithKline, Teva, and Novartis. Dr. Daley-Yates is an employee and shareholder of GlaxoSmithKline. Dr. Hermann received financial support for research from APEPTICO LABORATORIOS CINFA, S.A.; MEDA Pharma, Sun Pharma Advanced Research Company Ltd., Takeda, and Zentiva. Dr. Dissanayake is a full-time employee of Mundipharma Research Limited. Dr. De Backer is founder/shareholder of FLUIDDA. Dr. Horhota is an employee of Boehringer-Ingelheim.

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