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METHODS IN CLINICAL PHARMACOLOGY

Comparison of systemic pharmacodynamic effects of two combination pressurized metered dose inhalers that deliver salmeterol and fluticasone propionate

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AIM

The aim of this study was to test the systemic pharmacodynamic effects of the salmeterol component of two pressurized metered dose inhalers that delivered a combination of salmeterol and fluticasone propionate (SM/FP).

METHODS

This was a six-way crossover study in 43 adult subjects, using a single blind design (subject blinded to product and clinical assessor blinded for all measurements). Each subject received single doses of two, six, and twelve inhalations from test and reference products that delivered SM/FP as 25/125 mcg per inhalation. Heart rate, QTcB, and plasma potassium and glucose were monitored over 6 h.

RESULTS

Safety equivalence was shown by relative potency analysis for primary endpoints of maximum heart rate and maximum QTcB, since the 90% confidence intervals for both endpoints were within the acceptance limit of (0.67, 1.50). There were six secondary analyses for relative potency and equivalence was met for five of these endpoints. There were also 18 pairwise comparisons performed at each dose level. No statistical differences (95% confidence intervals included zero) among these pairwise comparisons were seen at the two-inhalation dose (therapeutic dose) or the six-inhalation dose. At the supratherapeutic dose of twelve inhalations, the test product was either comparable to or statistically less than that of the reference product for all comparisons. Overall, the results demonstrated comparable systemic safety. No differences were seen between the products in reported adverse events.

CONCLUSION

The safety equivalence of the systemic pharmacodynamic effects of the SM component of the test and reference SM/FP products was demonstrated.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A pressurized metered dose inhaler (pMDI) combination product of salmeterol/fluticasone propionate (SM/FP) has recently been approved in the UK as therapeutically equivalent to the innovator product.
- Pharmacodynamic study is frequently conducted to supplement pharmacokinetic data in EU therapeutic equivalence regulatory submissions of inhalation products.

WHAT THIS STUDY ADDS

- This research describes a safety equivalence study that was pivotal in the approval of a new SM/FP inhalation product.
- Results of this clinical study demonstrated the equivalent safety of the SM component of a new pMDI combination product of SM/FP with that of the innovator pMDI combination product.

Introduction

A combination product of the long-acting **beta-2 agonist salmeterol** and the **glucocorticoid fluticasone propionate** (SM/FP) in an HFA-143a-containing pressurized metered dose inhaler (pMDI) was approved for the treatment of patients with asthma in the UK in 2000. A subsequent product (test product) was approved in May 2015 as the first HFA-134a-containing pMDI of SM/FP in the UK that used the Committee for Medicinal Products for Human Use Guideline on the Requirements for Clinical Documentation for Orally Inhaled Products (EMA-OIP Guideline) [1] to demonstrate therapeutic equivalence to the reference product. Prior attempts to develop a SM/FP combination product as a pMDI [2] or as a dry powder inhaler [3], had been unsuccessful.

The purpose of this article is to report on the results of a pivotal systemic pharmacodynamic study that was part of this test product approval. Studies to document the *in vitro* pharmaceutical performance (which showed comparable performance for most measures) and *in vivo* bioequivalence of the test product (which showed bioequivalence for most measures) are presented elsewhere [4, 5].

According to the EMA-OIP Guideline [1], bioequivalence of the test and reference products must be demonstrated for the pharmacokinetic parameters of maximum plasma concentration (C_{max}) and area under the plasma concentration *vs*. time curve (AUC) for each drug of a combination product. The pharmacokinetic programme conducted on each of two strengths of the SM/FP test product (25/125 and 25/250) included the examination of the systemic absorption of product in the presence of oral charcoal, in the absence of charcoal, and when administered with a holding chamber. Bioequivalence of FP was established, but there were a few instances where a pharmacokinetic parameter for SM was slightly above the 125% confidence interval acceptance limits for the bioequivalence parameter (C_{max} of 126% (low strength), C_{max} of 130% (high strength), AUC of 133% (low strength)). In a Scientific Advisory Meeting with the Medicines and Healthcare products Regulatory Agency (MHRA), it was agreed that demonstration of the safety of the SM levels of the test products would be required and that a pharmacodynamic relative potency assessment would be an acceptable method for investigating equivalence with respect to the systemic safety of SM. Relative potency, defined as the ratio of the potency of the test product to that of the reference product, reflects the relationship between the dose response curves of the test and reference products. The EMA-OIP Guideline states "Therapeutic equivalence in respect of safety should be demonstrated by investigation of bioequivalence based on pharmacokinetic data, relevant cardiovascular, biochemical and physiological parameters, and monitoring of adverse events." With regard to SM, it was proposed that pharmacodynamic equivalence with respect to safety could be assessed with the following endpoints: the maximum change from baseline for the cardiovascular parameters of heart rate and QTcB and for the biochemical parameters of potassium (greatest decrease from baseline) and glucose (maximum change from baseline). The selection of these endpoints was based on literature studies which demonstrated that these measurements are able to show a dose response with SM [6–8]. The MHRA agreed with these endpoints and further recommended that the measurement of relative potency for these endpoints should be conducted for the pair of doses which lay on the steepest linear portion of the dose response curve, to maximize the sensitivity of the analysis.

This report describes a systemic pharmacodynamic study that was designed to examine the safety criteria discussed above for the SM component of the test product in healthy subjects. Although the EMA-OIP Guideline suggests that systemic pharmacodynamic studies should include patients with asthma, the MHRA agreed at a Scientific Advice Meeting with the sponsor that if adequate justification could be provided, healthy adult subjects would be a suitable population for this study [9]. There are several advantages of using healthy subjects for a pharmacodynamic study with respect to safety. Healthy subjects are a more homogeneous population and can be selected as naïve to both drugs, avoiding confounding use of non-study-related drugs. Healthy subjects are also better able to inhale the medication than patients with asthma, aiding generation of a homogeneous data set. Since pharmacodynamic equivalence is relative and depends on the same baseline characteristics from week-to-week, a better and more reliable comparison could be obtained with healthy subjects. This rationale was discussed with the MHRA at the Scientific Advice Meeting on 8 September 2010, and it was agreed that healthy subjects would be a suitable population.

Methods

Products

The test product was Sirdupla™ manufactured by 3M UK Plc (UK) and marketed by Mylan N.V. (UK). The reference

product was a commercial batch of Seretide™ Evohaler™ manufactured and marketed by GlaxoSmithKline plc (UK). Both products delivered 25 mcg SM (as salmeterol xinafoate) and 125 mcg FP per actuation.

Subjects

Inclusion criteria. Healthy, non-smoking male and female subjects, aged 18–55 years (inclusive) with a forced expiratory volume in 1 s of ≥85% of predicted normal and a body mass index of 22–27 kg m^{-2} (males) or 19–24 kg m^{-2} (females) met the inclusion criteria. All subjects were required to be using an adequate method of contraception from admission through 12 weeks after last administration.

Exclusion criteria. Exclusion criteria were evidence or history of clinically significant abnormalities or disease or chronic respiratory disorders, taking of any non-prescription (except paracetamol) or prescription (except contraceptives) medication within 2 or 4 weeks prior to dosing, respectively, and females being pregnant, nursing or lactating.

Ethics

The protocol was reviewed and approved by an Independent Ethics Committee and by the MHRA. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice. All subjects were required to give written informed consent. The trial was registered on Clinicaltrials.gov (NCT02232087).

Study design

This was a single-centre, six-period, randomized, crossover study in healthy subjects. Each dose period was separated by 5–14 days. The study was conducted using a single blind design (subject blinded to device and clinical assessor blinded for all measurements). A double-dummy design was not possible because of the inability to prepare a placebo pMDI matching the reference product. However, impartiality of the data collection/analysis was maintained as a label was wrapped around the inhaler actuator to hide each product logo in order to blind the subject to the identity of the inhaler being administered, and all study assessments following dosing were performed by clinic staff who did not participate in the dosing during any study period and who were therefore blind to the treatment.

Each subject was trained in the proper breathing and device use techniques and received doses of two, six, and twelve inhalations from both test and reference products that delivered 25 mcg SM and 125 mcg FP per inhalation. For a successful dose to provide evaluable pharmacodynamic (PD) data, a subject had to demonstrate good inhalation technique as instructed with no visible aerosol cloud for both inhalations of the two-inhalation dose, for at least five of the inhalations for the six-inhalation dose, and for at least ten of the inhalations for the twelve-inhalation dose.

Electrocardiogram (ECG) heart rate and QTcB interval (applying Bazett's correction for heart rate) were measured at 30 min pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 h post-dose. Plasma potassium and glucose levels were measured at 60 and 30 min pre-dose and at 0.5, 1, 2, 4, and 6 h post-dose.

The primary endpoints for each subject were maximum heart rate and maximum QTcB interval over 0–6 h post-dose adjusted for baseline. The secondary endpoints (adjusted for baseline) for each subject were heart rate and QTcB intervals at 15 and 30 min and 1 h post-dose; plasma potassium level at 4 and 6 h post-dose, and minimum plasma potassium level over 0–6 h post-dose; plasma glucose level at 30 min and 1 h post-dose, and maximum plasma glucose level over 0–6 h post-dose, and area under the curve from time zero to 6 h (AUC 0–6 h) for heart rate, QTcB, plasma potassium and glucose levels.

Assessments

Twelve-lead electrocardiograms (ECGs) were recorded in triplicate, approximately 2–4 min apart, and the average of these ECG measurements served as each subject's recorded heart rate and QTcB values. Plasma concentrations of potassium and glucose were measured using standard clinical chemistry tests.

Safety

Adverse events were recorded throughout the study by investigator questioning and by spontaneous reporting. Clinical chemistry and haematology parameters were assessed at screening and at the last visit of each subject. Vital signs (systolic and diastolic blood pressure and heart rate measurements) were measured prior to every dose. Recordings of 12-lead ECG (QT interval, QTcB interval, PR interval, QRS interval and heart rate) were measured at screening and over 6 h during all study days.

Number of subjects planned

It was planned to enrol 48 subjects into the study, in order to obtain data from approximately 42 evaluable subjects for the primary PD analysis. An evaluable subject for relative potency analyses was defined as having provided data for the primary parameters at all three dose levels of both the test and reference products. Assuming similar variability to that of similar internal studies and a true test *vs.* reference relative potency of 1.1, at least 42 evaluable subjects were considered sufficient to have approximately 90% power to conclude relative potency, i.e., 90% confidence intervals (CIs) for relative potency of the primary endpoints to be contained within the acceptance limit of (0.67, 1.50). Replacement of discontinued subjects was permitted to achieve the required number of evaluable subjects.

Statistical analyses

Relative potency. A two-step analysis was performed to determine the best doses pairs to assess the estimates of relative potency. In the first step, a mixed model analysis of variance (ANOVA) was performed for each pairwise selection of doses with baseline value (pre-dose on the visit in question), treatment (test or reference product), sequence, visit, dose on a log scale, and the interaction of log dose and treatment as fixed effects and subject within sequence as a random effect. The test of parallelism (i.e. the

treatment by log dose interaction) of the dose pairs for the test and reference products was assessed and a *P*-value of ≥0.10 was required. The interaction terms for all pairwise dose comparisons were non-significant (i.e. the *P*-value for the interaction term for all dosing pairs was ≥0.10 for all parameters). Therefore a second analysis of variance was performed removing the interaction term from the model. This second analysis tested the additional assumptions necessary for determination of relative potency. These assumptions were a significant dose response, i.e. the *P*-value for log dose was ≤0.05 between at least one pairwise selection of doses, and a non-significant difference between test and reference treatments was required, i.e. the *P*-value for the test treatment had to be ≥0.05 for that pairwise selection of doses.

The measurement of relative potency was conducted for the pair of doses which met the above criteria and lay on the steepest linear portion of the dose–response curve. The log estimate of relative potency (log ρ) was obtained as the ratio of the estimated treatment effect as measured by the difference in the intercepts of the parallel lines (β_2) divided by the estimate of the common slope for log dose (β_1):

$$\log \rho = \beta_2/\beta_1.$$

The 90% CI for the log of relative potency was estimated based on Fieller's theorem [10]. The estimated relative potency and its 90% CI was then calculated by exponentiation of the log of relative potency and its CI. The potency of the test and reference product for a PD parameter was considered equivalent if the 90% CI for the estimate of relative potency was completely contained within the limits of 0.67–1.50.

Pairwise comparisons. For each dose, the 95% CIs for the mean difference in the change from baseline for the test product *vs*. the reference product were calculated for all primary and secondary PD endpoints. PD parameters were analysed using a mixed model analysis of covariance (ANCOVA) in order to obtain the treatment comparisons for each dose. The model included treatment (i.e. test or reference product), sequence, visit, dose as a categorical variable, interaction of treatment and dose and the baseline value (pre-dose) as fixed effects and subject nested within sequence as a random effect. Point estimates, *P*-values and 95% CIs for the mean difference between test and reference products at each dose level were constructed using the error variance obtained from the ANCOVA. No statistical difference was observed in a pairwise comparison if the 95% CI included zero.

The relative potency analysis used data from subjects who had evaluable data for both the test and reference products for each of the doses relating to a dosing pair (i.e. two and twelve inhalations; two and six inhalations; or six and twelve inhalations). The response scale ANCOVA included all subjects who had evaluable PD data for both test and reference products for at least one dose level. Between 41 and 44 subjects met this criteria for each evaluation.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [11], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [12, 13].

Results

Population

Overall, 52 subjects, including four replacement subjects, were randomized into the study. Nine subjects (17.3%) were discontinued from the study. Three subjects withdrew consent, three subjects were discontinued due to poor inhalation technique, two subjects had a QTc interval >500 ms (a protocol subject withdrawal criterion), and one subject discontinued due to adverse events. The remaining 43 subjects completed the study and received all three dose administrations of both study products.

The relative potency analysis used data from subjects who had evaluable data for both the test and reference products for each of the doses relating to a dosing pair (i.e. two and twelve inhalations; two and six inhalations; or six and twelve inhalations). The response scale ANCOVA included all subjects who had evaluable PD data for both test and reference products for at least one dose level. One additional subject was excluded from one analysis because of physical activity that inadvertently affected the ECG evaluation. The data from at least 41 subjects and up to 44 subjects met the acceptance criteria and were included in each parameter analysis.

Response profiles

The pharmacodynamic responses between the test and reference products for heart rate, QTcB, plasma potassium level and plasma glucose level were comparable over the 6 h evaluation interval. No apparent difference in the test and reference responses for any dose at any time point was seen. For the evaluation of the response profiles, all available data were included. Thus, 43 subjects were included for the two inhalations dose of both products, 44 subjects were included for the six inhalations and twelve inhalations dose of the test product and the twelve inhalations dose of the reference product, and 45 subjects were included in the six inhalations dose of the reference product. Figure 1 shows results for heart rate and QTcB, and Figure 2 shows results for plasma concentrations of potassium and glucose.

Relative potency

The two-step analysis allowed the identification of the possible dose pairs to assess the estimates of relative potency and the selection of the dose pair with the steepest dose response. An example of the two-step analysis for the primary parameters is given in Table 1. Dose-related increases in heart rate, QTcB and plasma glucose, and decreases in plasma potassium were noted following treatment with both test and reference products as indicated by at least one pair of doses achieving

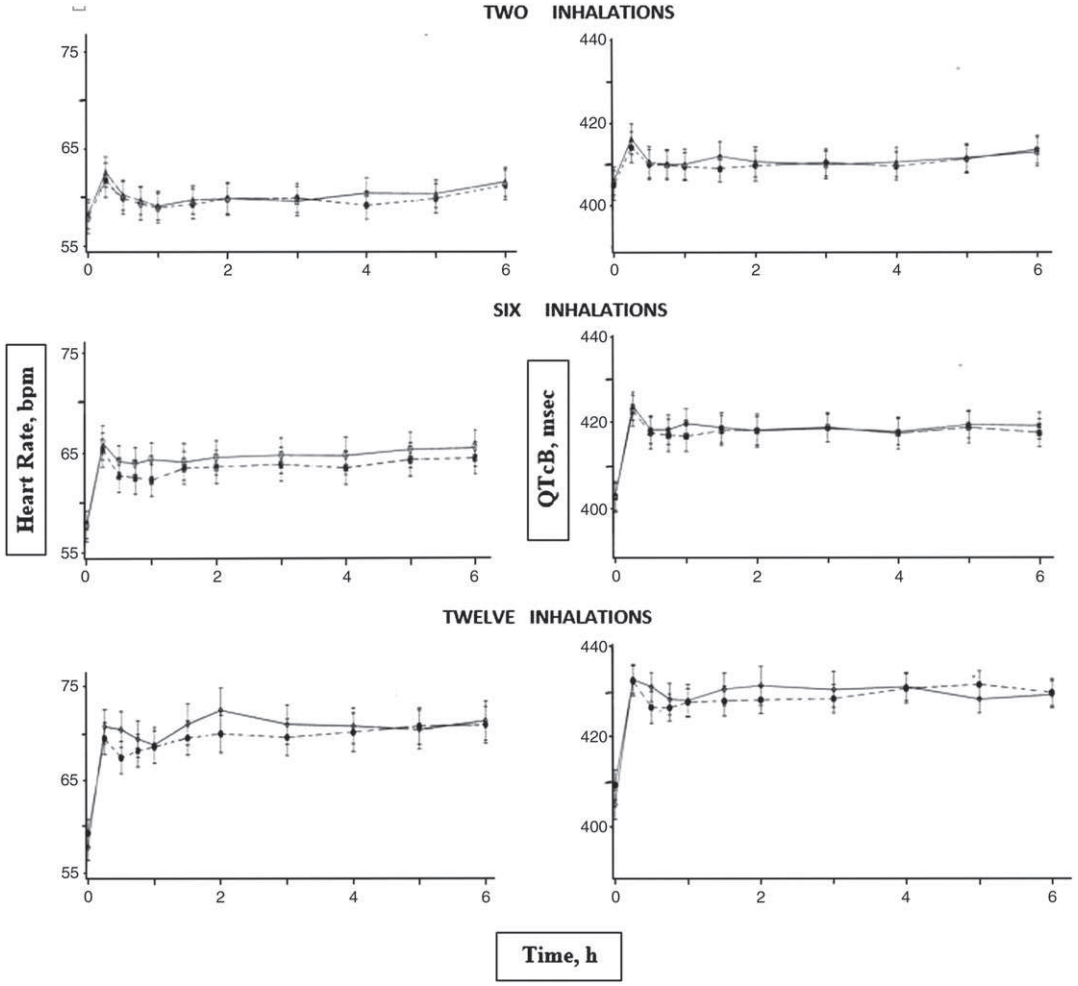

Figure 1

Heart rate and QTcB values (mean ± SE) in subjects given doses of 2, 6 and 12 inhalations. Dashed lines = test product; solid lines = reference product

$P < 0.001$ for the log dose term in the ANCOVA (i.e. statistically significant dose response).

Equivalence was demonstrated by the relative potency analysis of both primary endpoints of maximum heart rate and maximum QTcB (Table 2). There were six secondary analyses for relative potency and equivalence was met for five of the six secondary endpoints. Equivalence was not demonstrated for maximum plasma glucose concentration; however, this result was not necessarily indicative of a systematic difference between test and reference products on plasma glucose as the relative potency for plasma glucose AUC (0–6 h) met the equivalence criteria.

Clinical endpoint pairwise comparisons

There were 18 pairwise comparisons performed at each dose level. Table 3 presents the results for all doses. The table shows that no statistical difference was seen for any comparison at the recommended therapeutic two inhalations dose. Similarly, no statistical differences among these pairwise comparisons were seen at the six inhalations dose.

Only at the supratherapeutic dose of twelve inhalations were statistical differences observed, but only for six of the eighteen pairwise comparisons (Table 3). For each comparison, the test product was statistically less than that of the reference product.

Safety

Twenty-two subjects (42.3%) experienced at least one adverse event (AE) during the study and 19 subjects (36.5%) experienced an AE related to a study product. There were no serious AEs. Three subjects (5.8%) had AEs that led to discontinuation. Two of the subjects were discontinued due to prolonged QT interval (pre-specified in the protocol), one subject after dosing with twelve inhalations of test product and one subject after dosing with twelve inhalations of reference product; and one subject discontinued due to unrelated AEs of headache and upper respiratory tract infection after dosing with two inhalations of reference product.

One subject (2.1%) had a severe AE of presyncope approximately 2 h after receiving six inhalations of test product,

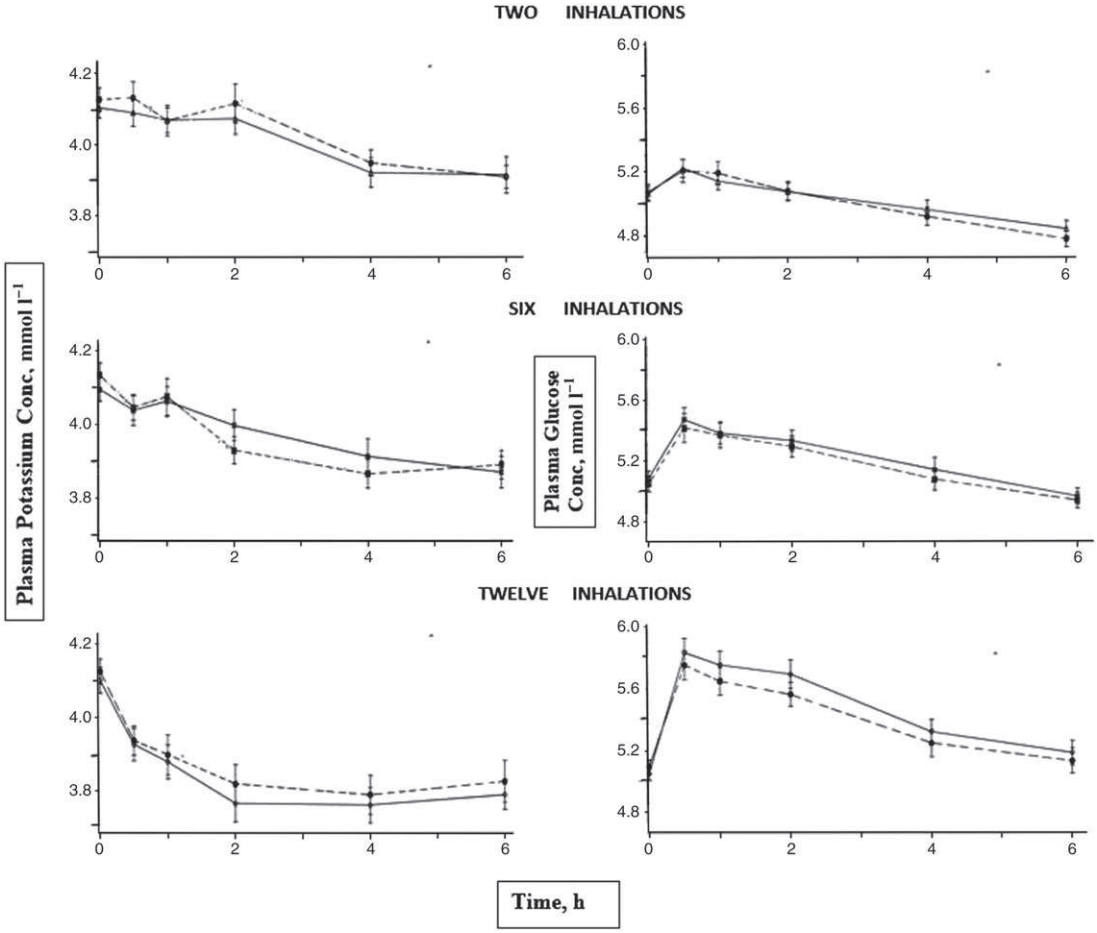

Figure 2

Plasma concentrations of potassium and glucose (mean ± SE) in subjects given doses of 2, 6 and 12 inhalations. Dashed lines = test product; solid lines = reference product

Table 1

Two-step analysis to determine the best dose pairs to assess the estimates of relative potency for the primary parameters

Primary parameter	Test *N*	Ref *N*	Dose pair, inhalations	*P*-value treatment by log dose	*P*-value dose response	*P*-value treatment difference	Steepness of dose response
Maximum heart rate	42	42	2 and 6	0.70	<0.001	0.51	0.1809
	43	43	2 and 12	0.38	<0.001	0.34	0.1403
	42	42	6 and 12[a]	0.66	<0.001	0.13	0.3181
Maximum QTcB	42	42	2 and 6[a]	0.81	<0.001	0.65	0.1110
	43	43	2 and 12	0.12	<0.001	0.32	0.0637
	42	42	6 and 12	0.15	<0.001	0.19	0.1094

[a]Dose pair selected for dose response

which was assessed as possibly related; and one subject (2.1%) had an unrelated AE of moderate severity (upper respiratory tract infection) 10 days after receiving two inhalations of reference product; this subject was discontinued before receiving the next dose due to AEs of headache and upper respiratory infection. All other AEs were mild in severity.

The incidences of AEs and study product-related AEs were similar for the test and reference products at each dose level and there were no notable differences between the test and reference products. There was a notable dose-related trend in the incidence of AEs. For both products, the incidence of study product-related AEs increased with the number of

Table 2

Relative potency analyses of the primary and secondary endpoints

Endpoint	Parameter	Dose pair (inhalations)	Relative potency	90% CI
Primary	Maximum heart rate	6 and 12	0.8619	0.7209, 1.0128
	Maximum QTcB	2 and 6	1.0760	0.8206, 1.4238
Secondary	AUC heart rate	6 and 12	0.8665	0.7304, 1.0122
	AUC QTcB	2 and 6	1.0279	0.8266, 1.2809
	Minimum potassium	6 and 12	1.0020	0.8152, 1.2314
	AUC potassium	6 and 12	1.0056	0.7526, 1.3451
	Maximum glucose	6 and 12	0.8000	0.6255, 0.9808
	AUC glucose	6 and 12	0.8349	0.6814, 0.9973

inhalations. Overall, the most commonly reported AEs were headache, dizziness, feeling hot and nervousness, which were all reported by three or more subjects during the study.

No laboratory test results or vital signs measurements were considered clinically significant and reported as AEs.

Discussion

The goal of this study was to demonstrate the safety of the SM component of the SM/FP test product and ultimately to support the SM pharmacokinetic data that did not meet the bioequivalence acceptance criteria. EMA-OIP Guideline specifies that safety must be established by demonstrating that the safety parameters of the test product are either comparable to or less than the corresponding reference product parameters. The EMA-OIP Guideline suggests two analyses to fulfil this safety requirement for a PD study. First the relative potency must show equivalence, and in this study equivalence was established for both primary endpoints and for five of the six secondary endpoints. Secondly, the clinical endpoint comparisons demonstrate comparability, and again comparability (i.e. no statistical difference) was seen for all pairwise comparisons at the therapeutic dose of two inhalations or at six inhalations, and was comparable or less than that of the reference product at twelve inhalations.

Thus, the present pharmacodynamic study was successful in demonstrating that the SM component of the combination SM/FP product delivered *via* a new pMDI test product is not associated with increased systemic PD effects compared with that delivered by the reference pMDI. By demonstrating equivalent safety of the SM component, these results supplemented the *in vivo* pharmacokinetic bioequivalence data and, taken together with the *in vitro* data, formed an acceptable approval package to establish the therapeutic equivalence of the test product to the reference SM/FP pMDI product.

It is assumed that the pharmacodynamic and safety effects observed in this study were primarily due to the effects of SM in the products [14]. At the therapeutic dose of two inhalations, 50 mcg SM was delivered and the observed systemic pharmacodynamic effects (heart rate, QTcB interval, plasma potassium and plasma glucose) of both the test and reference products were expected based on the known pharmacology of SM and were found to be equivalent. At higher doses the test product produced the expected dose-related beta-agonist pharmacodynamic effects; these effects were either comparable to or slightly less than those produced by the reference product. Given that pharmacokinetic studies have shown that the systemic absorption of the SM component of the test product produced blood levels that were bioequivalent to or in a few cases higher than that of the reference product, any observations of lower PD effects with the test product could not be due to lower SM absorption. It therefore seems reasonable to conclude that the test product was as safe and well-tolerated as the reference product based on the PD measurements of this study. The present study appears narrow in focus and ignores the contributions of FP. However, for product approval, the EMA-OIP Guidance only requires the demonstration of bioequivalence in pharmacokinetic studies of each strength under several conditions (with charcoal, without charcoal, and with the assistance of a holding chamber). Failing to meet the bioequivalence acceptance criteria for either component of a combination product could require additional PD study; in the present case a few SM bioequivalence measures were slightly higher than the upper limit of the acceptance criteria, raising issues of safety for the SM component. The present study's objective was to evaluate any safety concerns of these SM plasma levels and was designed in consultation with the MHRA. As there were no safety concerns with the FP component, PD effects contributed by FP were not considered in the study design.

It is recognized that this pharmacodynamic study included healthy subjects rather than patients with asthma. The selection of healthy subjects rather than a patient group for a PD study has been debated in scientific/regulatory meetings and the consensus (but not uniform agreement) is that the best design to discriminate product differences when comparing equivalence is to include healthy subjects as they are the less variable pharmacokinetic population than patients with compromised lung function and are therefore the preferred study population [8, 15].

In conclusion, this study has demonstrated the equivalent safety of the systemic pharmacodynamic effects of the SM component of the test and the reference SM/FP products.

Table 3

Pairwise comparisons

Parameter	Dose[a]	Diff[b]	95% CI	*P*-value	Compar-ability	Parameter	Dose[a]	Diff[b]	95% CI	*P*-value	Compar-ability
Heart Rate at 15 min (bpm)	2	−0.6	(−2.5, 1.4)	0.55	Yes	AUC QTcB over 0–6 h (ms·h)	2	−0.27	(−21.99, 21.46)	0.98	Yes
	6	−0.6	(−2.5, 1.3)	0.53	Yes		6	−0.68	(−22.39, 21.02)	0.96	Yes
	12	−2.5	(−4.5, −0.6)	0.011	No		12	−19.28	(−40.90, 2.34)	0.08	Yes
Heart Rate at 30 min (bpm)	2	0	(−2.2, 2.1)	0.97	Yes	Plasma potassium at 4 h (mmol l^{-1})	2	0.01	(−0.09, 0.12)	0.79	Yes
	6	−1.2	(−3.4, 0.9)	0.27	Yes		6	−0.06	(−0.17, 0.04)	0.25	Yes
	12	−4.2	(−6.4, −2.1)	<0.001	No		12	0.02	(−0.09, 0.13)	0.71	Yes
Heart Rate at 1 h (bpm)	2	0	(−2.1, 2.2)	0.96	Yes	Plasma potassium at 6 h (mmol l^{-1})	2	−0.02	(−0.13, 0.09)	0.72	Yes
	6	−2	(−4.2, 0.1)	0.063	Yes		6	0	(−0.11, 0.11)	0.99	Yes
	12	−1.3	(−3.5, 0.8)	0.23	Yes		12	0.04	(−0.07, 0.15)	0.5	Yes
Maximum Heart Rate over 0–6 h (bpm)	2	−0.2	(−2.6, 2.2)	0.87	Yes	Minimum plasma potassium over 0–6 h (mmol l^{-1})	2	0.04	(−0.03, 0.10)	0.26	Yes
	6	−0.9	(−3.3, 1.5)	0.45	Yes		6	0	(−0.06, 0.06)	0.93	Yes
	12	−2.4	(−4.8, 0.0)	0.053	Yes		12	0.02	(−0.04, 0.08)	0.48	Yes
AUC Heart Rate over 0–6 h (bpm·h)	2	−1.38	(−12.98, 10.22)	0.81	Yes	AUC plasma potassium over 0–6 h (mmol·h l^{-1})	2	0.085	(−0.341, 0.511)	0.69	Yes
	6	−4.9	(−16.49, 6.69)	0.41	Yes		6	−0.216	(−0.638, 0.205)	0.31	Yes
	12	−10.64	(−22.17, 0.88)	0.07	Yes		12	0.209	(−0.212, 0.631)	0.33	Yes
QTcB at 15 min (ms)	2	−1.3	(−5.7, 3.2)	0.58	Yes	Plasma glucose at 30 min (mmol l^{-1})	2	−0.023	(−0.164, 0.118)	0.75	Yes
	6	−0.8	(−5.3, 3.6)	0.7	Yes		6	−0.021	(−0.161, 0.118)	0.77	Yes
	12	−3.7	(−8.1, 0.8)	0.11	Yes		12	−0.13	(−0.270, 0.009)	0.067	Yes
QTcB at 30 min (ms)	2	0.1	(−4.9, 5.1)	0.97	Yes	Plasma glucose at 1 h (mmol l^{-1})	2	0.039	(−0.096, 0.174)	0.57	Yes
	6	−0.3	(−5.3, 4.6)	0.89	Yes		6	0.023	(−0.111, 0.156)	0.73	Yes
	12	−7.9	(−12.9, −2.9)	0.002	No		12	−0.155	(−0.289, −0.022)	0.023	No
QTcB at 1 h (ms)	2	0.2	(−4.7, 5.1)	0.94	Yes	Maximum plasma glucose over 0–6 h (mmol l^{-1})	2	−0.007	(−0.168, 0.154)	0.93	Yes
	6	−2.6	(−7.5, 2.2)	0.28	Yes		6	−0.065	(−0.224, 0.093)	0.42	Yes
	12	−3.4	(−8.3, 1.5)	0.17	Yes		12	−0.143	(−0.302, 0.016)	0.078	Yes
Maximum QTcB over 0–6 h (ms)	2	0.9	(−3.9, 5.7)	0.71	Yes	AUC plasma glucose over 0–6 h (mmol·h l^{-1})	2	−0.151	(−0.7464, 0.4434)	0.62	Yes
	6	0	(−4.7, 4.7)	1	Yes		6	−0.041	(−0.6293, 0.5473)	0.89	Yes
	12	−5.9	(−10.6, −1.1)	0.016	No		12	−0.733	(−1.3224, −0.143)	0.015	No

[a]Number of inhalations
[b]Test – Reference

Competing Interests

This study was sponsored by 3M UK Plc. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: L.I.H., V.S. and C.W. are employees of 3M UK Plc; J.E. received scientific advisory grants from 3M UK Plc; D.C. and P.L. are employees of Quotient Clinical, the contract research organization that performed the study and declare that they received no financial support from any organization for the submitted work.

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Contributors

L.I.H. and J.E. designed the study. V.S. and L.I.H. contributed to study execution. D.C. and C.W. were responsible for PD data analyses. P.L. was the principal investigator of the study. P.L. and J.E. contributed to the safety monitoring. All authors assisted in the interpretation of the data, participated in discussions and provided critical review of all drafts of the manuscript and provided their final approval of all content.

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