Original Research

Ethnic Sensitivity Study of the Extrafine, Single-Inhaler, Triple Therapy Beclomethasone Dipropionate, Formoterol Fumarate, and Glycopyrronium Bromide Pressurized Metered Dose Inhaler in Japanese and Caucasian Healthy Individuals: A Randomized, Double-Blind, Single-Dose Crossover Study

Massimo Cella, PhD[1]; Jörg Täubel, MD[2,3]; Irisz Delestre-Levai, MD, PhD[1]; Anne Tulard, PhD[1]; Andrea Vele, MSc[1]; and George Georges, MD[1]

[1]Global Clinical Development, Chiesi Farmaceutici SpA, Parma, Italy; [2]Richmond Pharmacology, London, United Kingdom; and [3]St. George's University, London, United Kingdom

ABSTRACT

Purpose: A number of single-inhaler, fixed-dose, triple combinations are available for the management of chronic obstructive pulmonary disease and/or asthma. One of these is the extrafine formulation beclomethasone dipropionate, formoterol fumarate, glycopyrronium bromide (BDP/FF/GB). Given that differences in ethnicity can result in differences in systemic exposure, we evaluated the relative pharmacokinetic (PK) profiles of BDP/FF/GB in Japanese vs Caucasian healthy volunteers to assess the need for dose adjustment.

Methods: This randomized, double-blind, single-dose, 4-way crossover study recruited healthy men and women 20 to 55 years of age; for each Japanese person a Caucasian was enrolled who matched in terms of sex, age, and weight. Study treatments included BDP/FF/GB 200/12/25 and 400/12/25 μg (therapeutic), 800/48/100 μg (supratherapeutic), and placebo. PK blood samples were taken up to 24 hours for evaluation of BDP, beclomethasone 17-monopropionate (B17MP, an active metabolite of BDP), and formoterol and up to 48 h for GB. The primary objective was to characterize the PK profiles of BDP, FF, and GB after administration of a single dose of BDP/FF/GB in Caucasian and Japanese healthy volunteers in terms of the AUC_{0-t} and C_{max} of B17MP, formoterol, and GB.

Findings: Of the 32 recruited participants (16 Japanese and 16 Caucasian), 30 completed the study. A clear plasma exposure dose-response relationship was found for all 4 molecules. B17MP C_{max} geometric mean ratios for Japanese vs Caucasian participants for the 3 study treatments ranged from 1.17 to 1.26, and AUC_{0-t} ratios ranged from 1.16 to 1.22; thus, the findings were comparable between the ethnicities. Formoterol exposure was higher in Japanese than Caucasian participants (C_{max}, 1.22–1.53; AUC_{0-t}, 1.23–1.40). The GB C_{max} with BDP/FF/GB 400/12/25 μg (1.09) and AUC_{0-t} values for all three doses (0.98–1.17) were comparable in the 2 populations, but C_{max} with 200/12/25 and 800/48/100 μg were higher in Japanese participants (1.32 and 1.42, respectively). Pharmacodynamic (cortisol, potassium, glucose, blood pressure, heart rate, and QT interval with the Fridericia correction) and safety profile results were similar in the 2 ethnicities, with most patients not experiencing any adverse events.

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Implications: Exposure to BDP/FF/GB pressurized metered dose inhaler at therapeutic and supratherapeutic doses was associated with higher plasma levels in Japanese versus Caucasian healthy volunteers. These PK differences did not translate into meaningful differences in the safety or pharmacodynamic parameters assessed in this study and were consistent with the results of other long-term (52-week) published studies. Dose adjustments in Japanese people are not deemed necessary.

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Key words: ethnic sensitivity, inhaled corticosteroid, long-acting β_2-agonist, long-acting muscarinic antagonist, pharmacodynamics, pharmacokinetics.

INTRODUCTION

A number of single-inhaler, fixed-dose, triple combinations are available for the management of chronic obstructive pulmonary disease (COPD) and/or asthma. By delivering all 3 molecules simultaneously, such combinations are easier for patients than using multiple inhalers (often with different instructions for use) and therefore may contribute to improved adherence. One of these single-inhaler, fixed-dose combinations is the extrafine formulation beclomethasone dipropionate (BDP), formoterol fumarate (FF) and glycopyrronium bromide (GB). Extrafine particles are more able to consistently reach small airways than nonextrafine particles,[1–3] with resulting enhanced drug delivery to these areas, improved overall lung deposition, and lower deposition in the oropharynx.[4] The efficacy and tolerability of BDP/FF/GB via pressurized metered dose inhaler (pMDI) has been evaluated in five 52-week studies in patients with asthma or COPD.[5–8] These studies were predominantly conducted in Caucasian populations.

Significant ethnic differences can exist in the 4 pharmacokinetic (PK) phases of a drug substance, especially in metabolism, for which both genetic and environmental factors have been implicated,[9] resulting in varying degrees of difference in systemic exposure.[10] These differences are particularly relevant for East Asian countries (including China, Korea, and Japan), leading to a reluctance to rely on clinical data from other ethnicities for drug approval. Therefore, we conducted a study to evaluate the relative PK profiles of BDP/FF/GB in Japanese versus Caucasian healthy volunteers to assess the potential need for dose adjustment. We also assessed pharmacodynamic and safety profiles.

PARTICIPANTS AND METHODS

Study Design

This was a randomized, double-blind, single-dose, 4-way crossover study. After a screening visit, eligible participants were admitted to the clinic on 4 occasions, with each clinic visit lasting from the day before dosing until 48 hours after dosing and separated by at least 21 days. Participants were randomized according to a balanced block scheme using a randomization list to 1 of 4 sequences, with every individual receiving each of the study treatments in randomized order. The 4 study treatments were 2 therapeutic doses of BDP/FF/GB (200/12/25 and 400/12/25 μg), a supratherapeutic dose of BDP/FF/GB (800/48/100 μg), and placebo, administered double-blind as 2 inhalations from each of 4 matching pMDIs. BDP/FF/GB 200/12/25 μg was administered as 2 inhalations of 100/6/12.5 μg plus 6 of placebo, BDP/FF/GB 400/12/25 μg as 2 inhalations of 200/6/12.5 μg plus 6 of placebo, and BDP/FF/GB 800/48/100 μg as 8 inhalations of 100/6/12.5 μg. The placebo pMDI was identical to the active pMDIs in terms of presentation, appearance, and excipients, with the only difference being the exclusion of the active ingredients; the success of the matching was not evaluated.

All participants fasted from 10 hours before dosing until 4 hours after dosing, subsequently receiving standard meals until discharge. No fluid intake was permitted from 75 minutes before dosing until 1 hour after dosing, with participants then required to consume 240 mL of water every 2 hours for the following 6 hours. Participants were to avoid strenuous activities from 24 hours before dosing until discharge and alcohol or caffeine intake from 48 hours before dosing until discharge.

At each visit PK blood samples were taken at intervals up to 24 hours for evaluation of BDP, beclomethasone 17-monopropionate (B17MP, an active metabolite of BDP), and formoterol and up to 48 hours after dosing for GB. Blood was collected in K_2-EDTA tubes and stored at≤–20°C until submitted

for analysis of BDP and B17MP levels in plasma using a validated LC/MS-MS method, with limits of quantitation of 10.0 pg/mL for BDP and 20.0 pg/mL for B17MP (Appendix). For the formoterol and GB evaluations, samples were collected in lithium heparin tubes, stored at≤−65°C, and analyzed using validated LC/MS-MS methods, with limits of quantitation of 1.00 pg/mL for both (Appendix). Urine samples were collected up to 48 hours after dosing for evaluation of GB and up to 24 hours for free and total (free plus conjugated) formoterol, with samples stored at≤−65°C and then analyzed using validated methods (Appendix). Pharmacodynamic blood samples were taken up to 4 hours after dosing for glucose and potassium and up to 24 hours for cortisol, with samples stored at −20°C until analyzed using photometry for glucose, potentiometry for potassium, and a validated ultrahigh performance LC/MS-MS method for cortisol (Appendix). ECG (using 12-lead digital Holter) and vital signs assessments were recorded up to 24 hours after dosing. The study was registered at ClinicalTrials.gov (NCT03859414). There were no substantial protocol amendments. The study was approved by an independent ethics committee and was performed in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization notes for guidance on Good Clinical Practice (ICH/CPMP/135/95).

Study Population

The study recruited healthy men and women 20 to 55 years of age with a body mass index of 18 to 25 kg/m^2 who were nonsmokers or ex-smokers (<5 pack-years). The intention was to recruit at least 7 women and 7 men per ethnicity; for each Japanese person, a Caucasian was to be enrolled who matched in terms of sex, age (±5 years), and weight (±15%). The Japanese participants had to have been born in Japan, with all 4 grandparents who were Japanese, and to have resided outside Japan for no more than 5 years (cumulative). No other drug therapy was permitted from 14 days before entry (other than occasional acetaminophen or ibuprofen, hormonal contraceptives, and hormone replacement therapy for postmenopausal women). Individuals were excluded if they had heavy caffeine consumption (>28 cups per week of coffee or similar caffeinated beverages) or who were night shift workers. Full inclusion and exclusion criteria are listed in the Appendix. All participants provided written informed consent before any study-related procedure.

Outcomes

The primary objective was to characterize the PK profiles of BDP, FF, and GB after administration of a single dose of BDP/FF/GB in Caucasian and Japanese healthy volunteers in terms of the C_{max} and AUC_{0-t} of B17MP, formoterol, and GB. Secondary PK variables were as follows: BDP: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $t_{½}$; B17MP and formoterol: t_{max}, $AUC_{0-\infty}$, and $t_{½}$; GB: t_{max}, AUC_{0-24} and AUC_{0-48} after dosing, $AUC_{0-\infty}$, and $t_{½}$; formoterol (total and free) in urine: cumulative urinary excretion from 0 to 4 hours (Ae_{0-4}), 4 to 12 hours (Ae_{4-12}), 12 to 24 hours (Ae_{12-24}), and 0 to 24 hours (Ae_{0-24}) after dosing, fraction of dose excreted (fe), and renal clearance (CLr); and GB in urine: Ae_{0-4}, Ae_{4-12}, and Ae_{12-24}, cumulative urinary excretion from 24 to 48 hours (Ae_{24-48}) and 0 to 48 hours (Ae_{0-48}) after dosing, fe, and CLr.

In addition, the general tolerability and the pharmacodynamic properties of BDP/FF/GB were assessed as secondary objectives. The pharmacodynamic variables were as follows: plasma cortisol: AUC_{0-24} and C_{min}; serum potassium: AUC_{0-4} and C_{min}; serum glucose: AUC_{0-4} and C_{max}; blood pressure: diastolic and systolic AUC_{0-24} normalized by time (AUC_{0-24}/24); and Holter-extracted 12-lead ECG: peak and average 24 hours heart rate and QTc interval corrected using the Fridericia calculation (QTcF) at peak and at 24 hours.

Statistical Analysis

The study was not formally powered. A total of 32 individuals (16 per ethnicity) was deemed sufficient to assess the objectives of the study. PK and pharmacodynamic (potassium, glucose, and cortisol) analyses were performed using WinNonlin Phoenix, version 8.0 (Pharsight Corporation, Palo Alto, California). AUC_{0-t}, AUC_{0-4}, AUC_{0-24}, and AUC_{0-48} were computed using the linear trapezoidal rule, and $AUC_{0-\infty}$ was calculated as the sum of AUC_{0-t} and a residual extrapolated to infinite time. C_{max}, C_{min}, t_{max} and fe (the fraction of dose, expressed in percentage, excreted unchanged in urine after dosing) were obtained directly from the experimental data without interpolation, with $t_{½}$ calculated from the individual drug concentrations versus time profiles. Ae (expressed in weight) was calculated from the concentration in urine and the volume of urine, and CLr was calculated as Ae/AUC,

Table I. Baseline demographic characteristics of the study participants.*

Characteristic	Japanese (n = 16)	Caucasian (n = 16)
Age, y	30.7 (7.5)	30.9 (7.2)
Male sex, No. (%)	8 (50.0)	8 (50.0)
Weight, kg	58.5 (8.36)	62.3 (8.78)
Body mass index, kg/m^2	21.3 (1.63)	21.4 (1.90)
Smoking status, No. (%)		
Nonsmoker	15 (93.8)	12 (75.0)
Ex-smoker	1 (6.3)	4 (25.0)

* Data are presented as mean (SD) unless otherwise specified.

for a period of 24 hours for FF and 48 hours for GB. For the main PK analyses, treatment groups were compared by descriptive analyses using the ratio of the geometric means.

The safety population comprised all randomized participants who received at least 1 dose of study drug. The PK data were analyzed in the PK population, which was the safety population excluding those without any valid PK measurement or with major protocol deviations. Pharmacodynamic data were analyzed in the pharmacodynamic population, which was all patients in the safety population except those without any valid pharmacodynamic measurement or with major protocol deviations that significantly affected pharmacodynamic properties.

RESULTS

Study Participants

The study was conducted between March 18 and July 24, 2019, in a specialist research unit in the United Kingdom. Of the 32 recruited participants (16 Japanese and 16 Caucasian), 30 completed the study. Both participants who prematurely discontinued the study were Japanese, with 1 withdrawing because of a protocol deviation (testing positive for drug abuse) and 1 because of a viral upper respiratory tract infection. The demographic characteristics of the 2 groups were similar (as would be expected given the matching of recruitment for sex, age, and weight) (Table I); the mean weight of the Caucasian participants was 6.5% greater but within the 15% variance specified in the protocol.

Pharmacokinetics

Mean plasma concentration vs time profiles by dose level in the Japanese and Caucasian participants are shown in Figure 1A to D. A clear dose-response relationship was found across doses for B17MP (Figure 1A); BDP was rapidly metabolized such that values were below the limits of quantitation across much of the evaluation period (Figure B). In addition, dose-response relationships were found across the 2 formoterol doses and the 2 GB doses (in that the higher dose clearly separated from the lower dose administered in the 200/12/25 and 400/12/25 μg groups) (Figure 1C and D).

The B17MP C_{max} and AUC_{0-t} values were generally comparable between the Japanese and Caucasian participants across BDP/FF/GB doses, as indicated by the geometric mean ratios (Tables II and III). Formoterol exposure was higher in the Japanese participants than in the Caucasian participants. For GB, C_{max} after inhalation of the 200/12/25 and 800/48/100 μg doses was again higher in Japanese participants than in Caucasian participants, whereas the C_{max} for 400/12/25 μg and the AUC_{0-t} values for all 3 doses were generally comparable in the 2 populations.

The secondary plasma exposure parameters were generally consistent with the primary parameters in that exposure in the Japanese participants was slightly higher than in the Caucasian participants (Table III). The t_{max} for the 4 molecules was similar across BDP/FF/GB doses and was similar between the 2 populations. The $t_{½}$ values for BDP, B17MP, and formoterol were also similar across doses and between

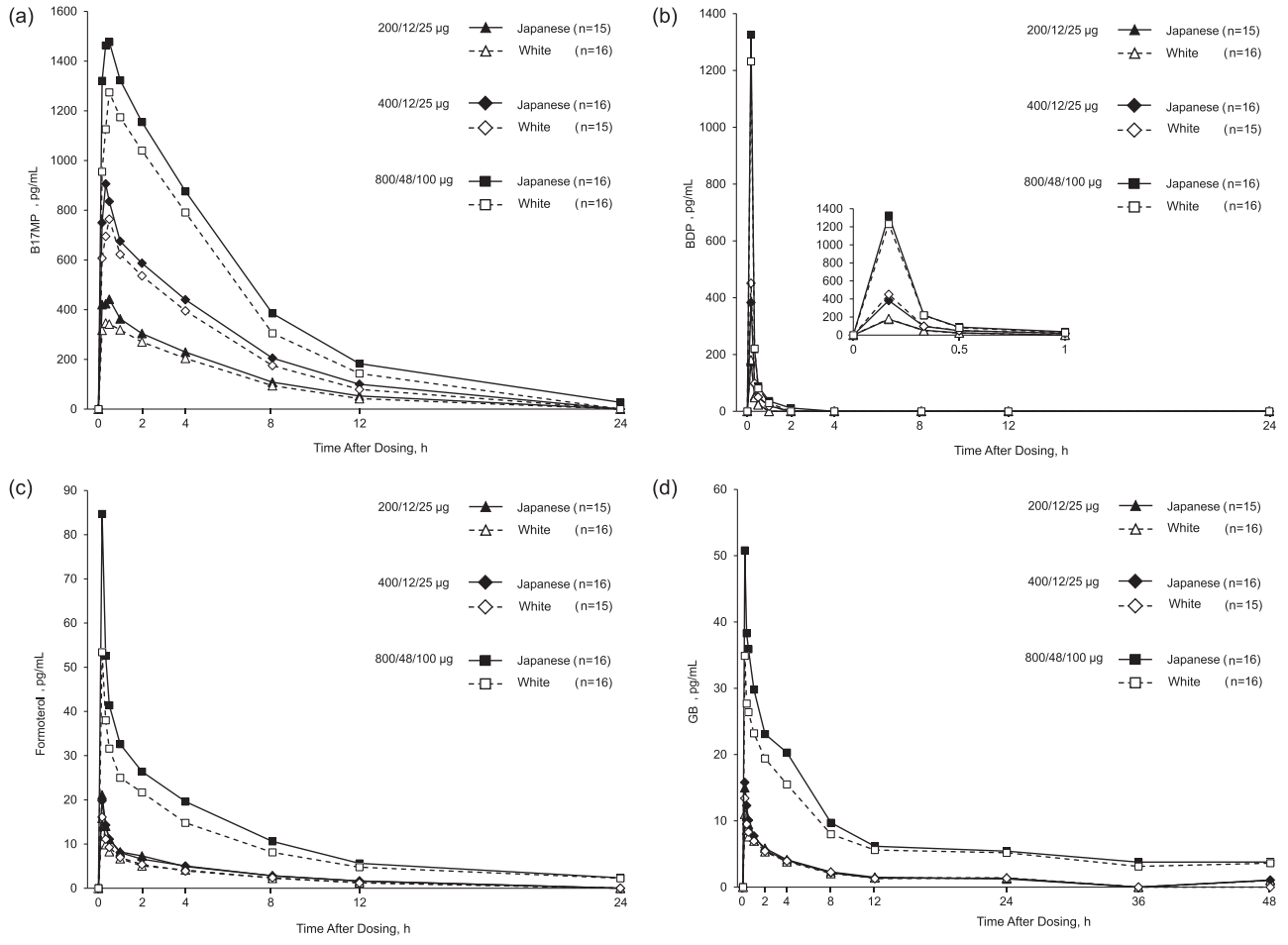

Figure 1. Mean plasma concentration–time profiles for (A) beclomethasone 17-monopropionate (B17MP), (B) beclomethasone dipropionate (BDP), (C) formoterol, and (D) glycopyrronium bromide (GB) (pharmacokinetic population).

Table II. Geometric mean ratios for Japanese vs Caucasian participants (pharmacokinetic population).

Dose	B17MP		Formoterol		GB	
	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}
Therapeutic dose 1: 200/12/25 μg	1.26	1.16	1.36	1.40	1.32	1.06
Therapeutic dose 2: 400/12/25 μg	1.19	1.22	1.22	1.23	1.09	0.98
Supratherapeutic dose: 800/48/100 μg	1.17	1.22	1.53	1.29	1.42	1.17

B17MP = beclomethasone 17-monopropionate; GB = glycopyrronium bromide.

the 2 populations. Too few participants had GB $AUC_{0-\infty}$ and $t_{½}$ assessed for reliable conclusions to be drawn (0–1 participants for $AUC_{0-\infty}$ and 2–6 for $t_{½}$).

Total formoterol urine PK parameters were slightly lower in the Japanese participants than the Caucasian participants, whereas the free formoterol and GB parameters were generally slightly higher in the Japanese population (Table IV).

Pharmacodynamic Properties

A BDP dose-related suppression of plasma cortisol was observed in terms of both AUC_{0-24h} and C_{min}, with no difference between the 2 ethnicities (Table V). Serum potassium was similar with the 2 therapeutic doses and placebo in terms of AUC_{0-4} and C_{min}, with no effect of ethnicity; the supratherapeutic dose slightly decreased both values, with a similar effect in the 2 groups. Furthermore, levels of serum glucose were similar with the 2 therapeutic doses and placebo; the supratherapeutic dose increased levels in both ethnicities, with slightly higher values in the Japanese group.

There was no evidence of a treatment-related effect on blood pressure (diastolic or systolic), and results were similar for the 2 ethnicities. Dose-related but not clinically significant increases were found in mean heart rate in both cohorts, with all treatments (including placebo) at the 10-minute postdose timepoint, then decreasing to baseline for the 200/12/25- and 400/12/25-μg doses; values with the supratherapeutic doses remained above baseline up to 8 hours after dosing for Caucasian participants and up to 12 hours after dosing for Japanese participants. Mean HR_{0-24} was slightly higher in Japanese participants than in the Caucasian participants with all treatments (including placebo). Similarly, dose-related increases in mean QTcF interval values were observed in both Japanese and Caucasian participants, with early peak values after administration of BDP/FF/GB that then returned to baseline. Differences between the 2 populations were not clinically relevant.

Table III. Summary of plasma pharmacokinetic parameters (pharmacokinetic population).*

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)
BDP						
C_{max}, pg/mL	175 (71.9)	203 (104)†	367 (188)	448 (217)	1326 (772)	1243 (803)
t_{max}, h	0.17 (0.17, 0.18)	0.17 (0.17, 0.17)†	0.17 (0.17, 0.23)	0.17 (0.17, 0.18)	0.17 (0.17, 0.17)	0.17 (0.15, 0.17)
AUC_{0-t}, h·pg/mL	45.1 (23.4)	50.8 (28.4)†	103 (54.1)	116 (57.3)	321 (185)	293 (185)
$AUC_{0-\infty}$, h·pg/mL	59.3 (3.58)‡	84.2 (17.4)§	122 (56.2)‖	151 (48.2)#	332 (194)**	415 (168)††
$t_{½}$, h	0.310 (0.0107)‡	0.282 (0.0199)§	0.400 (0.152)‖	0.367 (0.128)#	0.516 (0.241)**	0.515 (0.0652)††
B17MP						
C_{max}, pg/mL	490 (158)	398 (164)	926 (349)	790 (312)	1638 (820)	1344 (550)
t_{max}, h	0.33 (0.17–2.00)	0.50 (0.17–4.00)	0.33 (0.17–2.00)	0.50 (0.17–2.00)	0.44 (0.17–2.00)	0.50 (0.50–2.05)

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Table III. (*continued*)

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)
AUC_{0-t}, h·pg/mL	2236 (429)	1941 (409)	4642 (1063)	3895 (1211)	9336 (2266)	7785 (2320)
$AUC_{0-\infty}$, h·pg/mL	2529 (467)	2180 (514)[†]	4955 (1012)	4243 (1216)	9652 (2213)	8087 (2271)
$t_{½}$, h	3.80 (0.485)	3.54 (0.594)[†]	4.14 (0.975)	3.68 (0.818)	4.22 (0.636)	3.79 (0.891)
Formoterol						
C_{max}, pg/mL	21.5 (9.40)	16.3 (8.12)	19.4 (8.93)	16.0 (7.30)	85.2 (46.1)	52.5 (23.3)
t_{max}, h	0.17 (0.17–2.00)	0.17 (0.17–1.00)	0.17 (0.17–0.33)	0.17 (0.17–1.00)	0.17 (0.17–1.00)	0.17 (0.15–0.50)
AUC_{0-t}, h·pg/mL	55.7 (11.5)	42.7 (17.4)	54.3 (13.1)	44.9 (12.4)	262 (72.3)	205 (56.1)
$AUC_{0-\infty}$, h·pg/mL	67.1 (10.9)[‡‡]	63.2 (19.1)[§§]	71.1 (15.7)[#]	57.5 (7.19)[§§]	287 (81.8)	228 (66.0)[‖‖]
$t_{½}$, h	4.96 (0.804)	5.20 (0.924)[†]	5.21 (1.03)[‖‖]	5.71 (1.91)[†]	7.12 (1.18)	8.16 (1.76)
GB						
C_{max}, pg/mL	15.1 (9.52)	11.5 (7.01)	15.9 (11.6)	13.8 (8.80)	52.5 (35.4)	36.0 (25.4)
t_{max}, h	0.17 (0.17–4.00)	0.17 (0.17–2.02)	0.17 (0.17–0.50)	0.17 (0.17–1.00)	0.17 (0.17–2.00)	0.50 (0.15–4.00)
AUC_{0-24}, h·pg/mL	62.8 (25.1)	58.4 (24.0)	61.6 (28.5)	59.0 (24.0)	267 (97.9)	220 (88.3)
AUC_{0-48}, h.pg/mL	84.1 (42.0)	78.9 (38.2)	82.8 (42.4)	79.9 (39.6)	366 (148)	310 (133)
AUC_{0-t}, h.pg/mL	82.1 (44.1)	77.4 (39.9)	80.4 (43.7)	77.7 (41.3)	367 (148)	310 (133)

B17MP = beclomethasone 17-monopropionate; BDP = beclomethasone dipropionate; GB = glycopyrronium bromide; NC = not calculated.

[*] Data are presented as arithmetic mean (SD), except for t_{max}, which is median (range).

[†] n = 14.

[‡] n = 3.

[§] n = 6.

[‖] n = 13.

[#] n = 9.

[**] n = 12.

[††] n = 8.

[‡‡] n = 8.

[§§] n = 7.

[‖‖] n = 15.

Table IV. Summary of urine pharmacokinetic parameters (pharmacokinetic population).*

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)
Total formoterol						
Ae_{0-4}, μg	0.834 (0.355)	0.928 (0.289)	0.856 (0.410)	0.937 (0.429)	3.33 (1.84)	3.33 (1.54)
Ae_{4-12}, μg	0.539 (0.174)	0.625 (0.219)	0.539 (0.183)	0.966 (1.52)	2.23 (1.04)	2.32 (0.694)
Ae_{12-24}, μg	0.192 (0.0709)	0.210 (0.0912)	0.188 (0.0647)	0.313 (0.432)	0.750 (0.315)	0.737 (0.327)
Ae_{0-24}, μg	1.56 (0.541)	1.76 (0.481)	1.58 (0.569)	2.22 (2.00)	6.31 (2.47)	6.39 (1.72)
fe, % dose	13.0 (4.51)	14.7 (4.01)	13.2 (4.74)	18.5 (16.7)	13.1 (5.15)	13.3 (3.58)
CLr, L/h	26.0 (13.8)	44.3 (25.4)	25.5 (9.59)	48.5 (48.1)	24.8 (10.2)	34.1 (13.9)
Free formoterol						
Ae_{0-4}, μg	0.428 (0.156)	0.314 (0.123)	0.437 (0.184)	0.272 (0.128)	1.61 (0.773)	1.11 (0.446)
Ae_{4-12}, μg	0.254 (0.0982)	0.193 (0.0800)	0.241 (0.0751)	0.186 (0.0597)	0.928 (0.475)	0.759 (0.254)
Ae_{12-24}, μg	0.112 (0.0397)	0.102 (0.0492)	0.108 (0.0401)	0.102 (0.0489)	0.405 (0.168)	0.358 (0.166)
Ae_{0-24}, μg	0.794 (0.259)	0.608 (0.237)	0.786 (0.273)	0.561 (0.170)	2.94 (1.19)	2.22 (0.760)
fe, % dose	6.61 (2.16)	5.07 (1.97)	6.55 (2.27)	4.67 (1.41)	6.12 (2.48)	4.63 (1.58)
CLr, L/h	12.5 (3.91)	13.1 (3.31)	12.3 (3.10)	11.3 (3.19)	11.2 (3.55)	11.0 (2.94)
GB						
Ae_{0-4}, μg	0.687 (0.386)	0.505 (0.221)	0.706 (0.465)	0.474 (0.262)	2.76 (1.73)	1.84 (0.851)
Ae_{4-12}, μg	0.467 (0.223)	0.385 (0.145)	0.437 (0.205)	0.384 (0.117)	2.23 (1.30)	1.65 (0.576)
Ae_{12-24}, μg	0.360 (0.202)	0.293 (0.156)	0.345 (0.214)	0.288 (0.153)	1.51 (1.04)	1.12 (0.641)
Ae_{24-48}, μg	0.527 (0.295)	0.416 (0.246)	0.466 (0.331)	0.447 (0.218)	1.93 (1.37)	1.35 (0.804)
Ae_{0-48}, μg	2.04 (1.04)	1.60 (0.714)	1.95 (1.16)	1.59 (0.598)	8.42 (5.11)	5.97 (2.66)
fe, % dose	8.16 (4.18)	6.40 (2.86)	7.82 (4.63)	6.37 (2.39)	8.42 (5.11)	5.97 (2.66)

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Table IV. (*continued*)

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)
CLr, L/h	28.4 (16.5)	23.0 (8.11)	26.9 (14.3)	23.5 (9.90)	21.8 (6.79)	19.7 (4.29)

Ae = cumulative urinary excretion in stated period (in hours); CLr = renal clearance; fe = fraction of dose; GB = glycopyrronium bromide.

* Data are presented as arithmetic mean (SD).

Safety Profile

Most patients did not experience any adverse events, and of the adverse events that occurred, all were mild, with none resulting in withdrawal from the study or in death (Table VI). The only event to occur in more than 1 participant was headache, although this event was considered treatment related by the investigator in only 3 participants: in 1 Japanese participant and 1 Caucasian participant while receiving the supratherapeutic BDP/FF/GB dose and in 1 Caucasian participant while receiving placebo.

DISCUSSION

Given the PK factors that can be expected to potentially result in ethnic differences include protein binding, V_d, hepatic metabolism, and renal tubular secretion,[11] a range of factors could potentially result in ethnic differences in systemic exposure after drug inhalation. BDP is a prodrug that is rapidly hydrolyzed by tissue and blood esterase enzymes to B17MP.[12] Systemic absorption of unchanged BDP occurs mainly through the lungs with negligible oral absorption of the swallowed dose; systemic absorption of B17MP is both through the lungs and orally, with a high level of plasma protein binding (94%–96%). Subsequent enzymatic hydrolysis of BDP is followed by glucuronidation and rapid elimination mainly in the feces. After inhalation, FF is rapidly absorbed, with 61% to 64% protein binding, and then widely metabolized, primarily in the liver,[13] followed by urinary excretion.[14] In contrast, GB penetrates slowly through biological membranes (with a low apparent V_d)[15] and is then rapidly cleared by the systemic circulation before being excreted in the urine predominantly unchanged.[16]

Overall, after inhalation of single therapeutic and supratherapeutic doses of BDP/FF/GB, plasma exposure of all 4 molecules was slightly higher in Japanese healthy volunteers than in Caucasian healthy volunteers. Total formoterol urine PK parameters were lower in the Japanese participants than the Caucasian participants, whereas the free formoterol and GB parameters were generally higher in the Japanese population. However, any differences between the 2 populations in pharmacodynamic parameters were small and of short duration and therefore not clinically relevant. Furthermore, all BDP/FF/GB doses were well tolerated, with adverse events being mild and few considered related to treatment. Taken together, these findings suggest that the differences in systemic exposure between the 2 populations are not associated with any safety concerns, and therefore no dose adjustment is needed.

The increased systemic exposure to FF and GB in Japanese compared with Caucasian individuals that we observed in this study is consistent with a number of previous studies that evaluated a different GB formulation[17] and for the triple combinations of budesonide/FF/GB and indacaterol/glycopyrronium/mometasone furoate,[18,19] which supported the Japanese approval of a number of these therapies with no need for dose adjustment. For example, in a study that evaluated the PK properties of the different GB formulation, subsequently approved for the management of COPD in Japan, the geometric mean ratios of C_{max} and $AUC_{0–24}$ in Japanese versus

Table V. Summary of pharmacodynamic parameters (pharmacodynamic population).*

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg		Placebo	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)	Japanese (n = 14)	Caucasian (n = 16)
Plasma cortisol								
AUC_{0-24}, h·ng/mL	1545 (625)	1582 (425)	1306 (585)	1354 (259)	1214 (467)	1105 (257)	1678 (554)	1602 (291)
C_{min}, ng/mL	19.2 (14.8)	18.0 (7.27)	16.5 (14.9)	16.4 (6.40)	9.28 (6.07)	9.78 (4.44)	21.8 (10.7)	19.2 (5.96)
Serum potassium								
$AUC_{0-4,}$ h·mmol/L	16.8 (1.26)†	18.0 (1.15)	17.0 (1.07)	17.6 (0.734)	16.4 (2.64)‡	16.8 (1.20)	17.5 (1.22)	18.1 (0.943)
C_{min}, mmol/L	3.97 (0.197)	4.19 (0.209)	4.02 (0.214)	4.16 (0.172)†	3.69 (0.276)‡	3.91 (0.304)	4.12 (0.221)§	4.22 (0.306)‡
Serum glucose								
AUC_{0-4}, h·mmol/L	19.1 (1.15)	19.4 (1.79)	19.5 (1.24)	19.2 (0.862)	21.5 (1.97)	20.4 (1.44)	18.8 (1.19)	18.8 (0.850)
C_{max}, mmol/L	4.99 (0.335)	5.05 (0.665)	5.05 (0.338)	4.94 (0.246)	5.69 (0.637)	5.34 (0.413)	4.95 (0.469)	4.91 (0.236)
Blood pressure (AUC_{0-24}/24 h), mm Hg								
Diastolic	65.3 (4.1)	66.6 (4.8)	66.1 (3.9)	66.1 (5.0)	64.4 (3.4)	65.6 (4.6)	64.2 (3.8)	65.6 (5.4)
Systolic	102.0 (7.3)	106.2 (8.1)	103.1 (7.1)	105.5 (10.0)	102.5 (7.1)	106.2 (9.1)	99.6 (6.6)	104.5 (8.7)
Heart rate, beats/min								
Peak change from baseline in mean at 10 min	15.2 (15.6)	14.2 (8.1)	11.6 (9.8)	14.2 (13.6)	17.0 (8.7)	15.2 (11.1)	6.9 (9.5)	8.6 (8.5)
Average over 24 hours	70.3 (7.9)	61.8 (6.7)	71.6 (8.0)	61.8 (7.7)	75.1 (6.6)	65.8 (7.7)	68.2 (6.9)	60.4 (7.4)
QTcF interval, msec								
Peak change from baseline in mean	7.8 (3.6 to 12.0) at 20 min	8.3 (3.5 to 13.0) at 2 h	8.6 (4.3 to 13.0) at 4 h	7.9 (3.0 to 12.8) at 2 h	16.1 (10.1 to 22.2) at 1 h	17.1 (11.4 to 22.8) at 30 min	–7.7 (–13.7 to –1.7) at 8 h and 4.3 (–0.9 to 9.4) at 2 h	–1.5 (–5.0 to 2.1) at 10 min and 5.9 (2.2 to 9.5) at 2 h

(*continued on next page*)

Table V. (*continued*)

Parameter	Therapeutic Dose 1: 200/12/25 μg		Therapeutic Dose 2: 400/12/25 μg		Supratherapeutic Dose: 800/48/100 μg		Placebo	
	Japanese (n = 15)	Caucasian (n = 16)	Japanese (n = 16)	Caucasian (n = 15)	Japanese (n = 16)	Caucasian (n = 16)	Japanese (n = 14)	Caucasian (n = 16)
At 24 h	405.1 (395.2 to 415.0)	404.7 (394.3 to 415.1)	404.5 (397.1 to 411.8)	400.7 (390.0 to 411.5)	406.2 (398.7 to 413.6)	402.7 (393.4 to 412.0)	408.5 (396.5 to 420.4)	401.1 (392.2 to 409.9)

QTcF = QT interval corrected with Fridericia calculation.
* Data are presented as arithmetic mean (SD) except for QTcF, which is mean (90% CI) for change from baseline and mean (95% CI) at 24 hours.
† n = 14.
‡ n = 15.
§ n = 13.

Table VI. Number (percentage) of participants reporting adverse events (with preferred terms occurring in ≥2 participants with any treatment) (safety population).

Adverse Events	Japanese				Caucasian			
	200/12/25 μg (n = 15)	400/12/25 μg (n = 16)	800/48/100 μg (n = 16)	Placebo (n = 14)	200/12/25 μg (n = 16)	400/12/25 μg (n = 16)	800/48/100 μg (n = 16)	Placebo (n = 16)
Total adverse events	1 (6.7)	3 (18.8)	4 (25.0)	2 (14.3)	3 (18.8)	3 (18.8)	6 (37.5)	5 (31.3)
Headache	0	0	0	1 (7.1)	0	1 (6.3)	3 (18.8)	1 (6.3)
Treatment-related adverse events	0	0	1 (6.3)	1 (7.1)	0	0	3 (18.8)	1 (6.3)
Serious adverse events	0	0	0	0	0	0	0	0
Adverse events leading to study discontinuation	0	0	0	0	0	0	0	0
Adverse events leading to death	0	0	0	0	0	0	0	0

Caucasian healthy volunteers were 1.76 to 1.84 and 1.23 to 1.34, respectively, after administration of single 50-, 100-, or 200-μg doses.[17] Similarly, in a study that evaluated the PK properties of budesonide/FF/GB in Chinese, Japanese, and Caucasian healthy volunteers, systemic exposure to all 3 molecules after a single 320/9.6/18-μg dose was higher in the Japanese participants: the ratio of the unadjusted geometric means for $AUC_{0–12}$ and C_{max} was 1.32 and 1.41 for budesonide, 1.15 and 1.38 for formoterol, and 1.33 and 1.24 for GB, respectively, although with the authors concluding that the data indicated ethnic insensitivity.[18]

An important finding is that plasma exposure increased with increasing dose of all 3 molecules. For B17MP, C_{max}, $AUC_{0–t}$, and $AUC_{0–\infty}$ increased approximately dose proportionally between the 2 therapeutic doses in both ethnicities, as did $AUC_{0–t}$

between BDP/FF/GB 200/12/25 μg and the supratherapeutic dose, with C_{max} and $AUC_{0-\infty}$ increasing dose proportionally or slightly less. Similarly, the C_{max} and AUC_{0-t} of BDP increased dose proportionally between the 2 therapeutic doses; increases between BDP/FF/GB 200/12/25 μg and the supratherapeutic dose were more than dose proportional, but the rapid metabolism to B17MP perhaps makes these data less reliable. The 2 therapeutic doses contained the same dose of formoterol and GB; for these molecules, the increases between the therapeutic and supratherapeutic dose in C_{max} were dose proportional or slightly less than dose proportional in both ethnicities, whereas those in AUC values were overall dose proportional or more than dose proportional. Given that these assessments were performed after single doses (rather than at steady state) and in relatively small numbers of patients, the implications of this slightly more or less than dose proportionality are difficult to interpret, and this study was not designed to formally evaluate dose proportionality. However, the finding that exposure to formoterol and GB was generally similar after administration of the 2 therapeutic doses is important because it suggests that there is no drug-drug interaction between increased exposure to BDP/B17MP and exposure to FF and GB.

The pharmacodynamic and safety data further confirm the lack of ethnic sensitivity. Administration of inhaled corticosteroids, such as BDP, have a suppressive effect on the hypothalamic-pituitary-adrenal axis, resulting in a dose-dependent decrease in plasma cortisol.[20,21] We observed such decreases in the present study, with slight reductions compared with placebo in plasma cortisol AUC_{0-24} after administration of the 2 therapeutic doses and a greater reduction after administration of the supratherapeutic dose, with consistent effects in the 2 ethnicities. In addition, inhaled β_2-agonist bronchodilators can result in decreased potassium and increased glucose levels and increases in blood pressure, heart rate, and QTcF interval, although predominantly at supratherapeutic doses.[22] Again, we observed such changes in the present study, with small differences versus placebo after administration of the therapeutic FF dose and larger differences after administration of the supratherapeutic dose but with similar changes in the 2 ethnicities. Finally, most patients did not experience any adverse events, and of the adverse events that occured, all were mild.

The key strengths of the study are that by conducting it in a single center variations in analytical approaches and laboratories were avoided, with the crossover design meaning that patients acted as their own controls, again helping to reduce variability. The main limitations are that this was a single-dose study, so the parameters were not assessed at steady state. This approach may be why some of the increases in plasma exposure across doses were not dose proportional, although the study was not designed to evaluate dose proportionality. In addition, the analyses included data from a relatively small number of individuals. Furthermore, systemic exposure of an inhaled medication does not necessarily correlate with its topical efficacy but is more likely to be associated with tolerability. Finally, the single-dose nature of the study limits interpretation of the pharmacodynamic and safety data. In this context, the good overall safety and tolerability profiles of BDP/FF/GB in the 52-week studies is more informative[5–8] because these studies exposed large numbers of patients with asthma or COPD to long-term maintenance therapy.

CONCLUSIONS

In general, exposure to BDP/FF/GB pMDI at therapeutic and supratherapeutic doses was associated with elevation in some PK parameters in Japanese compared with Caucasian healthy volunteers. These PK differences did not translate into meaningful differences in safety and pharmacodynamic parameters and were consistent with the results of other published studies. As a consequence, dose adjustments in Japanese individuals are not deemed necessary.

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DISCLOSURE

Irisz Delestre-Levai was an employee of Chiesi, the sponsor of the trial, at the time it was conducted. Massimo Cella, Anne Tulard, Andrea Vele, and George Georges are employees of Chiesi. Jörg Täubel is employed by Richmond Pharmacology, a contract research organization paid to perform the study generating the data on which this publication is based. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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Address correspondence to: Massimo Cella, Global Clinical Development, Chiesi Farmaceutici SpA, Via Palermo, 26/A, Parma, 43100, Italy. E-mail: m.cella@chiesi.com.

SUPPLEMENTARY METHODS

BDP and B17MP evaluation in plasma

BDP, its metabolite B17MP and their internal standards were extracted from K_2EDTA human plasma by liquid/liquid extraction using a mixture of diethylether/hexane (40/60, v/v). The organic phase was evaporated and the residue reconstituted in injection solvent before injection in the UHPLC/MS-MS system (1290 Infinity II system equipped with G7167B well plate autosampler, G7116B column oven and G7120A binary pump, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 5500 Q-trap and API 6500, AB/MDS Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on a Kinetex C18 100 Å, 2.6 μm particle size, 100 × 3.0 mm I.D. column using mobile phase made of a mixture of mobile phase A: acetonitrile/high purity water (10/90, v/v) containing 2 mM ammonium formate and 0.05% trifluoroacetic acid and mobile phase B: acetonitrile/high purity water (90/10, v/v) containing 2 mM ammonium formate and 0.05% trifluoroacetic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst® (Ontario, Canada).

Calibration standards were prepared in bulk by spiking blank K_2EDTA human plasma with known amounts of BDP and its metabolite B17MP. Calibrator samples were frozen and kept below –20°C until used. Two sets of calibrators were then treated in exactly the same way as the samples to be assayed. The first set was injected at the beginning of the run and the second at the end of the run. The results of the daily QCs were acceptable, with means (CV %) of 30.9 (5.42), 461 (3.34) and 923 (3.69) pg/mL for BDP 30, 450 and 900 pg/mL, respectively, and 58.0 (4.69), 870 (3.74) and 1742 (3.78) pg/mL for B17MP 60, 900 and 1800 pg/mL, respectively.

Formoterol evaluation in plasma

Formoterol and its internal standard were extracted from acidified lithium heparinized human plasma by solid/liquid extraction on Oasis MCX, 30 μm, 10 mg, 96 well-plates. The eluates were evaporated and the residue reconstituted in injection solvent before injection in the UHPLC/MS-MS system (1290 Infinity II system equipped with G7167B autosampler, G7116B column oven and G7120A binary pump, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 6500 Q-trap and API 6500, AB/MDS Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on an Acquity UPLC HSS C18, 1.8 μm particle size, 50 × 2.1 mm I.D. column using mobile phase made of a mixture of mobile phase A: methanol/high purity water (10/90, v/v) containing 0.1% formic acid and mobile phase B: methanol/high purity water (90/10, v/v) containing 0.1% formic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst®, (Ontario, Canada).

Calibration standards were prepared in bulk by spiking blank acidified lithium heparinized human plasma with known amount of formoterol. Calibrator samples were frozen and kept below –65°C until used. Two sets of calibrators were then treated in exactly the same way as the samples to be assayed. The first set was injected at the beginning of the run and the second at the end of the run. The results of the daily QCs were acceptable, with means (CV %) of 2.83 (9.09), 57.8 (5.06) and 116 (4.59) pg/mL for formoterol 3, 60 and 120 pg/mL, respectively.

GB evaluation in plasma

GB and its internal standard were extracted from lithium heparinized human plasma by solid/liquid extraction on Oasis WCX, 30 μm 96 well microelution plate. The eluates were evaporated and the residue reconstituted in injection solvent before injection in the UHPLC/MS-MS system (1290 Infinity II system equipped with G7167B well plate autosampler, G7116B column oven and G7120A binary pump, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 4000, AB/MDS Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on an Acquity UPLC HSS T3, 1.8 μm particle size, 50 × 2.1 mm I.D. column using mobile phase that consisted of a mixture of mobile phase A: high purity water containing 0.1% formic acid and mobile phase B: acetonitrile containing 0.1% formic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst®, (Ontario, Canada).

Quality control samples were prepared in bulk prior to the start of the assay by spiking the matrix to be assayed with known amounts of GB. Quality control samples were frozen and kept below –65°C until used. The results of the daily QCs were acceptable, with means (CV %) of 3.01 (5.28), 74.8 (5.09) and 149 (6.34) pg/mL for GB 3, 75 and 150 pg/mL, respectively.

Formoterol evaluation in urine

Formoterol and its internal standard were extracted from human urine by solid/liquid extraction on Oasis HLB, 30 mg, 96 well-plate. The eluates were evaporated and the residue reconstituted in injection solvent before injection in the LC/MS-MS system (1100 series equipped with G1316A column oven, G1312A binary pump, and G1367A autosampler, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 4000 Q-Trap, AB Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on a Kinetex C18 100 A, 2.6 μm particle size, 50 × 3.0 mm I.D. column using mobile phase made of a mixture of mobile phase A: methanol/high purity water (10/90, v/v) containing 0.1% formic acid and mobile phase B: methanol/high purity water (90/10, v/v) containing 0.1% formic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst® (Ontario, Canada).

Quality control samples were prepared in bulk prior to the start of the assay by spiking the matrix with known amounts of formoterol. Quality control samples were frozen and kept below –65°C until used. The results of the daily QCs were acceptable, with means (CV %) of 27.7 (7.82), 292 (3.75) and 724 (3.78) pg/mL for free formoterol 30, 300 and 750 pg/mL, respectively, and 28.2 (12.43), 296 (7.49) and 720 (4.85) pg/mL for total formoterol 30, 300 and 750 pg/mL, respectively.

GB evaluation in urine

Glycopyrronium bromide and its internal standard were extracted from human BSA urine by solid/liquid extraction on Oasis WCX, 2 mg, 96 well microelution plate. The eluates were evaporated and the residue reconstituted in injection solvent before injection in the LC/MS-MS system (1290 Infinity system equipped with G1367A autosampler, G1316A column oven and G1312A binary pump, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 4000, AB/MDS Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on an Halo phenyl-hexyl, 50 × 2.1 mm I.D. column using mobile phase that consisted of a mixture of mobile phase A: acetonitrile/high purity water (10/90, v/v) containing 2 mM ammonium acetate and 0.05 % trifluoroacetic acid and mobile phase B: acetonitrile/high purity water (90/10, v/v) containing 2 mM ammonium acetate and 0.05 % trifluoroacetic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst®, (Ontario, Canada).

Calibration standards were prepared in bulk by spiking blank human BSA urine with known amount of GB. Calibrator samples were frozen and kept below –65°C until used. Two sets of calibrators were then treated in exactly the same way as the samples to be assayed. The first set was injected at the beginning of the run and the second at the end of the run. The results of the daily QCs were acceptable, with means (CV %) of 52.6 (4.66), 2058 (4.99) and 4241 (3.63) pg/mL for GB 50, 2000 and 4000 pg/mL.

Glucose and potassium evaluation in serum

Human serum samples were analyzed using Vitros microslide technology (Ortho Clinical Diagnostics, Turnhout, Belgium) based on colorimetric and potentiometric measurement principles. When serum comes into contact with dry chemical layers, reactions occur and are measured by the system. A colorimetric assay allows the determination of glucose in serum and a potentiometric assay allows the determination of potassium in serum. Glucose and potassium were detected using Vitros 250 analyzer (Ortho Clinical Diagnostics).

QC samples were prepared in bulk by diluting commercial performance verifier samples at two concentration levels. The results of the QCs were acceptable.

Cortisol evaluation in plasma

Cortisol and its internal standard were extracted from K_2EDTA human plasma by solid/liquid extraction on Oasis HLB 10 mg, 96 well-plate. The eluates were evaporated and the residue reconstituted in injection solvent before injection in the UHPLC/MS-MS system (1290 Infinity II system equipped with G7167B autosampler, G7116B column oven and G7120A binary pump, Agilent, Brussels, Belgium). The compounds of interest were detected by tandem mass spectrometry (API 4000, AB Sciex, Nieuwerkerk a/d Ijssel, The Netherlands). Separation was achieved on a Kinetex C18 100 Å, 100 × 3.0 mm I.D., 2.6 μm particle size column using mobile phase made of a mixture of mobile phase A: acetonitrile/high purity water (10/90, v/v) containing 2 mM ammonium acetate and 0.1% formic acid and mobile phase B: acetonitrile/high purity water (90/10, v/v) containing 2 mM ammonium acetate and 0.1% formic acid. Acquisition was performed using the Analyst^{TM} software of MDS Sciex Analyst®, (Ontario, Canada).

Calibration standards were prepared in bulk by spiking blank K_2EDTA human plasma with known amount of cortisol. Calibrator samples were frozen and kept below –20°C until used. Two sets of calibrators were then treated in exactly the same way as the samples to be assayed. The first set was injected at the beginning of the run and the second at the end of the run. The results of the daily QCs were acceptable, with means (CV %) of 15.3 (9.34), 306 (7.87) and 456 (6.36) ng/mL for cortisol 15, 300 and 450 ng/mL, respectively.

Inclusion criteria

1. Subject's written informed consent obtained prior to any study-related procedure.
2. Healthy male and female subjects aged 20 to 55 years, inclusive.
3. For Japanese subjects: subject had to be a Japanese subject who had resided outside Japan for no more than 5 years, born in Japan and holding a Japanese passport, with all four grandparents Japanese, as confirmed by interview.
4. Subject had to be willing and able to adhere to the prohibitions and restrictions specified in the protocol and able to comply with the correct use of the devices specified in the protocol.
5. Body mass index (BMI) within the range of 18.0 to 25.0 kg/m^2, inclusive.
6. Non-smokers or ex-smokers who smoked <5 pack-years (pack-years = the number of cigarette packs per day, times the number of years) and stopped smoking >1 year prior to screening.
7. Subject had to be healthy on the basis of physical examination, medical history, vital signs, and 12-lead ECG performed at screening and before randomization. If there were abnormalities, the subject could be included only if the investigator judged the abnormalities or deviations from normal to be not clinically significant. This determination had to be recorded in the subject's source documents and initialed by the investigator.
8. Vital signs within normal limits at screening and prior to randomization: DBP 60–90 mmHg, SBP 90–140 mmHg (duplicate measures performed after at least 5 min of resting; the mean value had to be within the defined range); pulse rate 40–110 bpm (duplicate measures performed after at least 5 min of resting; the mean value had to be within the defined range); and tympanic body temperature 35.5–37.5°C.
9. 12-lead digitized ECG (12-lead ECG) considered normal according to the investigator's judgment (120 msec ≤ time interval between the onset of the P wave and the beginning of the QRS complex [PR] ≤210 msec, time interval between the beginning of the Q wave and the termination of the S wave [QRS] ≤120 msec, QTcF ≤450 msec for males and QTcF ≤470 msec for females) at screening visit or prior to randomization.
10. Lung function measurements within normal limits at screening and prior to randomization: FEV_1 equal to or more than 80% of predicted normal value for the subject according to the Global Lung Function Initiative, European Respiratory Society (ERS) Task Force Lung Function Reference Values and FEV_1/forced vital capacity (FVC) ratio >0.70.
11. Women of proven non-childbearing potential (WONCBP) defined as physiologically incapable of becoming pregnant (i.e., post-menopausal or permanently sterile) and women of proven childbearing potential (WOCBP) fulfilling one of the following criteria:
    a. WOCBP with fertile male partners: they and/or their partner had to be willing to use a highly effective birth control method from the signature of the informed consent until the follow-up visit, or;
    b. WOCBP with non-fertile male partners (contraception was not required in this case).
12. Female subjects, except if postmenopausal, had to have a negative serum beta-human chorionic gonadotropin (HCG) test at screening and a negative urine pregnancy test on Day –1 prior to the first drug administration.
13. Subjects had to agree not to donate sperm or ova from the time of the first administration of study medication until 3 months after the end of the systemic exposure of the study drug or until the last follow-up visit, whichever occurred later.
14. Males fulfilling one of the following criteria:
    a. Males with pregnant or non-pregnant WOCBP partners: they had to be willing to use male condom from the signature of the informed consent until the follow-up visit, or;
    b. Non-fertile male subjects (contraception was not required in this case), or;
    c. Males with partner not of childbearing potential (contraception was not required in this case).

Inclusion criteria 7, 8, 9, and 12 were re-checked at randomization prior to the first study drug administration. In case inclusion criteria 8, 9, or 10 were not met at screening, these criteria could be re-checked once before randomization in a re-screening visit.

Exclusion criteria

Participation in another clinical study with an investigational drug in the 90 days or five half-lives of non-biological entities of that investigational drug (whichever is longer) preceding the administration of the study drug.

Clinically relevant and uncontrolled respiratory, cardiac, hepatic, gastrointestinal, renal, endocrine, metabolic, neurologic, or psychiatric disorders that could interfere with successful completion of this study.

Any other abnormal findings on vital signs, ECG, physical examination or laboratory evaluation of blood and urine samples that the investigator judged as likely to interfere with the study or pose an additional risk in participating.

Medical diagnosis of narrow-angle glaucoma, prostatic hypertrophy or bladder neck obstruction that in the opinion of the investigator could prevent use of anticholinergics.

History of asthma, including childhood asthma, COPD or any other chronic pulmonary diseases or conditions.

Positive human immunodeficiency virus 1 or 2 (HIV1 or HIV2) serology at screening.

Positive results from the hepatitis serology at screening which indicated acute or chronic hepatitis B (i.e., positive for hepatitis B surface antigen [HBsAg] and/or hepatitis B core antibody [anti-HBc]) or hepatitis C infection (positive for hepatitis C antibody).

Blood donation or blood loss (equal or more than 450 mL), less than two months prior to randomization.

Abnormal hemoglobin (Hb) level defined as <12.0 g/dL in females and <14.0 g/dL in males.

Positive urine test for cotinine at screening or prior to randomization.

Unsuitable veins for repeated venepuncture.

History or clinical evidence of drug and/or alcohol abuse within 12 months prior to screening and randomization.

Known intolerance/hypersensitivity to any of the excipients/components contained in any of the formulations used in the study.

Taking any drug treatment, including prescribed or over-the-counter (OTC) medicines as well as homeopathic remedies etc., in the 14 days before screening and prior to randomization, with the exception of:

Occasional paracetamol (maximum 3 g per day with a maximum of 10 g per 14 days for mild non-excluding conditions);

Hormonal contraceptives;

Hormonal replacement treatment for post-menopausal women;

Occasional ibuprofen (maximum 1.2 g per day, not to exceed 12 g in the 14 days before screening). Non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen were not allowed during the rest of the study.

Taking enzyme-inducing drugs, enzyme-inhibiting drugs, biologic drugs or any drugs known to have a well-defined potential for hepatotoxicity (e.g., isoniazide, nimesulide, ketoconazole) in the three months before screening or prior to randomization.

Heavy caffeine drinking >28 cups/week of coffee or similar caffeinated beverages (e.g., tea, cola).

Bacterial or viral respiratory tract, sinus or middle ear infection affecting health status within four weeks prior to randomization.

Night shift workers with night shifts within eight weeks prior to randomization and during the study.

Exclusion criteria 1, 8, 10, 12, 14, 15, 17, and 18 were re-checked at randomization prior to the first study drug administration. In case of out of-range values for exclusion criteria 3, 9, or 10 at screening that could indicate a temporary condition, these criteria could be re-checked once before randomization in a re-screening visit prior to the first study drug administration.