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Effect of zamicastat on blood pressure and heart rate response to cold pressor test: a double-blind, randomised, placebo-controlled study in healthy subjects

Marlene Fonseca[1], Cheila Ribeiro[2], Guillermo Fernández[3], Helena Gama[4], Luís Magalhães[2], Sara Carolina Henriques[1], Nuno Silva[1], Filipe Pinto[1], Luís Almeida[1], and Patricio Soares-da-Silva[5]

[1]BlueClinical Phase I
[2]Bial-Portela & C$^{\underline{a}}$ S.A.
[3]BIAL R&D Investments, S.A
[4]Bial-Portela & C$^{\underline{a}}$ S.A
[5]Faculty of Medicine

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Abstract

Aim: Inhibitors of dopamine-β-hydroxylase (DβH), such as zamicastat, emerged as promising drugs for pulmonary arterial hypertension (PAH). This study intended to validate the mechanism of action of zamicastat by studying its effect on the overdrive of sympathetic nervous system (SNS). Methods: This was a single-centre, prospective, double-blind, randomised, placebo-controlled, crossover study, with 400 mg zamicastat, in 22 healthy male subjects. Cold pressor test (CPT) was performed at screening and each treatment period at day 1 and day 10. The concentration of dopamine (DA), epinephrine (EPI), norepinephrine (NE) in plasma and 24h-urine, and DβH activity in plasma were measured. Results: For zamicastat compared to placebo, the difference between cold stimulus and rest phases on the change from baseline to day 10 of CPT showed an estimated decrease of -4.62 mmHg for systolic blood pressure (SBP; p=0.020). Zamicastat caused a decrease of -2.62 mmHg in mean arterial pressure (MAP) response to cold stimulus during CPT (p=0.025). At day 10, zamicastat elicited a statistically significant increase of 12.63 ng/L (p=0.040) and 19.22 ng/L (p=0.001) in plasma DA, before CPT and after CPT, and a significant estimated increase in plasma EPI change from baseline after CPT (p=0.040). Inhibition of plasma DβH activity ranged from 19.8% to 25.0%. At day 10, statistically significant reductions in 24-hour urinary excretion of EPI (p=0.002) and NE (p=0.001) were observed. Conclusions: Zamicastat decreased SBP and MAP response to cold stimulus during CPT, evidencing its effect on the overdrive sympathetic response to cold stimulus.

Introduction

Despite advances in treatment options for pulmonary arterial hypertension (PAH), the disease is still associated with worsening symptoms and increased mortality, so research for novel treatments persists.

The development of more effective therapeutic treatments has been recently focused on the hyperactivation of neurohumoral systems – essentially the sympathetic nervous system (SNS) – involved in the development and progression of hypertension and chronic heart failure. In fact, hyperactivation of SNS has been implicated in the progression of right heart failure and arrhythmia as premature ventricular contractions, ventricular arrhythmia, and sudden cardiac death. This involvement has been confirmed by both indirect (elevated plasma concentrations of catecholamines) and direct evidence, such as muscle sympathetic nerve activity, via microneurography of the peroneal nerve, and heart rate (HR) and baroreceptor reflex variability.

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In this context, the inhibition of the sympathetic nerve function with adrenoceptor antagonists appeared to be a promising approach to manage the hyperactivation of the SNS. However, most patients, particularly those with heart failure, do not tolerate the immediate hemodynamic deterioration that follows β-blocker treatment. An alternative strategy is to reduce the biosynthesis of norepinephrine (NE) through the inhibition of the enzyme dopamine-β-hydroxylase (DβH). This approach has several putative merits, such as gradual modulation, as opposed to abrupt inhibition of the sympathetic system, and increased availability of dopamine (DA), which can improve renal function. Therefore, it could be anticipated that inhibitors of DβH may provide significant clinical advantages in PAH patients over conventional adrenoceptor antagonists.

In this setting, zamicastat (also known as BIA 5-1058), a reversible DβH inhibitor developed by Bial-Portela & C$^{\underline{a}}$ S.A., emerged as an orally administered small molecule for the treatment of cardiovascular disorders, including PAH. It is a potent peripheric selective inhibitor of DβH, with limited access to the brain, that converts DA to NE in sympathetically innervated tissues, reducing the drive of the SNS. At this point, the confirmation of the mechanism of action of zamicastat in the SNS overdrive, through DβH inhibition, would validate and strengthen the efficacy and safety data collected so far.

Different methods for the evaluation of the autonomic nervous system (ANS) have been described, including the cold pressor test (CPT) and measurements of the levels of neurotransmitters. CPT is a simple, non-invasive validated test of sympathetic activation. It consists of a sympatho-excitatory stressor (cold) that activates afferent pain and temperature neurons, resulting in a centrally mediated stimulation of sympathetic efferent neurons. This induces vascular sympathetic activation, causing arteriolar constriction, increased HR, and increased cardiac contractility, with consequent increase in diastolic (DBP) and systolic blood pressure (SBP).

Measurements of plasma and urine catecholamines are a classic and reliable method for assessing ANS function. The concentration of plasma catecholamines has been shown to increase following various stressor stimuli, being an excellent tool to evaluate the impact of drugs on the SNS.

This study aimed to validate the proposed mechanism of action of zamicastat by studying its effects on the SNS, through the evaluation of the impact on blood pressure (BP) and HR response and assessment of plasma and urinary concentration of catecholamines and their metabolites, before and after CPT.

Materials and Methods

Study population

Study subjects were recruited from BlueClinical Phase I's pool of healthy volunteers and underwent a screening procedure comprising medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG), CPT, and clinical laboratory safety tests.

The main inclusion criteria were: healthy non-smoker or ex-smoker male subject with age [?]18 and [?]55 years; absence of clinically relevant diseases in medical history; resting seated SBP of 90-140 mmHg, DBP of 50-90 mmHg and pulse rate 45-100 bpm, inclusive; ability to tolerate the 3-minute cold stimulus phase of CPT; mean SBP increase of [?]7 mmHg during the cold stimulus phase of CPT; absence of relevant abnormalities on physical examination, 12-lead ECG, and clinical laboratory safety tests. The main exclusion criteria were: previous exposure to zamicastat; hypersensitivity and/or allergy reaction to the investigational medicinal product (IMP) or any of the excipients; any medical or surgical condition that may affect drug pharmacokinetics or subject safety; clinically relevant history of fainting, syncope, orthostatic hypotension, vasovagal reaction; history of alcoholism or drug abuse in the past two years; history of arterial hypertension, diabetes mellitus or Raynaud's phenomenon; orthostatic hypotension; QT interval corrected with Fridericia's formula (QTcF) $>$ 450 msec. Subjects who met all the inclusion criteria and none of the exclusion criteria at the screening visit were considered eligible for participation in the study.

Written informed consent was obtained from each prospective subject prior to any study procedure, after the physician provided all the information about the implications of participating in the study. Subjects were assured that they might abandon the study at any time without any prejudice.

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SARS-CoV-2 testing was performed prior to admission to each study period.

Study design and treatments

This was a single-centre, prospective, two-sequence, two-period crossover study, in which healthy male subjects were randomised to receive 400 mg zamicastat or placebo.

The study had a double-blind design and was placebo controlled to reduced potential bias. Compared with a parallel-group study, the crossover design reduces the influence of confounding covariates and provides more precise estimates of effects with fewer participants.

The study consisted of a screening evaluation, two treatment periods, and an end-of-study visit. In each treatment period, subjects were administered a 400 mg once-daily oral dose of zamicastat or matching placebo, for 10 days, in fed conditions. The two treatment periods were separated by a washout of at least seven days. Subjects were screened between days -28 to -3 (both inclusive) before the first IMP administration to confirm that they met the eligibility criteria for the study (Figure 1).

On both treatment periods, subjects were admitted to the clinical site two days before (day -2) the first IMP administration. On both treatment periods, the following procedures were completed on the day before (day -1) the first IMP administration: measurement of vital signs (BP, HR, and body temperature); 24-hour urine collection for catecholamines assay; collection of blood for plasma catecholamines concentration, DβH activity determination, and zamicastat and metabolites (BIA 5-453 and BIA 5-961) quantification; and CPT.

On the morning of days 1 to 10, subjects received 400 mg of zamicastat or matching placebo orally, after a moderate breakfast. On days 3, 6, 8, and 10, 24-hour urine was collected for catecholamines assay, and blood samples were collected for DβH activity determination and zamicastat and metabolites quantification. On day 1 and day 10, CPT was performed, and blood was collected for the determination of plasma catecholamines concentration, before and after CPT.

During the treatment days, the following safety assessments were performed: clinical laboratory safety test on day 5 (haematology and biochemistry); ECG on days 1, 3, 5, 7, and 9; and vital signs from day 1 to 10.

Cold pressure test

Each subject performed CPT at screening, day -1, and day 10 of each treatment period, approximately 3.5 hours after the start of a moderate breakfast. CPT was conducted approximately at the same time of the day, to reduce the impact of circadian variations of BP on the study outcomes. BP and haemodynamic variables were collected during the CPT using the Finapres® NOVA monitor (Finapres Medical Systems, Amsterdam). The CPT procedure was conducted using the Advanced Hemodynamics software application (Finapres Medical Systems, Amsterdam) to allow for the characterisation of further haemodynamic parameters. The following parameters were collected: SBP, DBP, HR, mean arterial pressure (MAP), cardiac output (CO), cardiac index (CIn), stroke volume (SV), SV index (SVI), total peripheral resistance (TPR), cardiac contractility (dP/dt) and left ventricular ejection time (LVET).

The test included three phases: i) rest phase (5 minutes), ii) cold stimulus phase (3 minutes), and iii) recovery phase (5 minutes). During the cold stimulus phase of CPT, subjects inserted their left hand up to the wrist in a temperature-controlled (4±0.5 ^{o}C) water bath, for three minutes.

The pharmacodynamics analysis set (PD population) included all subjects who received at least one dose of the IMP and had at least one valid post-dose for the corresponding PD assessment. Those subjects who belong to the PD set and did not deviate from the protocol in a way that might affect the evaluation of the corresponding PD variable were included in the per-protocol pharmacodynamics analysis set (PP-PD population).

Plasma and urinary catecholamines concentration

In each treatment period, for plasma catecholamines concentration assessment, venous blood samples (10 mL) were drawn into K_2EDTA tubes, on day -1 and day 10, 30±5 minutes before the rest phase of CPT

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and within 2 minutes after the recovery phase of CPT. The determination of the plasma-free fractionated catecholamines DA, epinephrine (EPI), and NE was performed by using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) of the method were 17.4 and 1116.8 ng/L, for DA, 17.6 and 1124.5 ng/L, for EPI, and 63.0 and 3966.2 ng/L, for NE, respectively.

For the determination of the urinary creatinine and catecholamines excretion profile, urine was collected for 24 hours, starting within 15 minutes before breakfast on days -1, 3, 6, 8, and 10, on each treatment period. The determination of urinary concentrations of total catecholamines and their metabolites was performed by high-performance liquid chromatography (HPLC) with electrochemical detection.

The catecholamines metabolites determined were: i) normetanephrine; ii) metanephrine; iii) 4-hydroxy-3-methoxy-phenylacetic acid, also named homovanillic acid (HVA); and iv) 4-hydroxy-3-methoxy-mandelic acid (HMMA), also named vanillylmandelic acid (VMA).

The 24-hour urinary catecholamines excretion profile was normalised by using the 24-hour creatinine concentration.

Δοπαμινε-β-ηψδροξψλασε (ΔβΗ) αςτιιτψ ασσαψ

For the determination of DβH activity in plasma, venous blood samples (6 mL) were drawn into lithium heparin tubes, 30 minutes after breakfast on day -1 and at pre-dose (within 10 minutes) on days 3, 6, 8, and 10.

The DβH activity assay relied on the conversion of tyramine to octopamine by DβH; octopamine was then determined in plasma, in accordance with the applicable principles of Good Laboratory Practices (GLP), using a previously validated LC-MS/MS analytical method. DβH activity was calculated as follows:

$$D\beta H\ activity\ \left(\frac{ng}{mL\ min}\right) = \frac{Octopamine\ concentration\ \left(\frac{\mu g}{mL}\right) \times 1000}{Incubation\ time: 45\ min}$$

The LLOQ and the ULOQ of the method for octopamine were 1.00 and 29.98 μg/mL, respectively.

Pharmacokinetic measurements

In each treatment period, for the determination of plasma concentrations of zamicastat and its metabolites, venous blood samples (6 mL) were drawn into lithium heparin tubes, 30 minutes after breakfast on day -1 and at pre-dose (within 10 minutes) on days 3, 6, 8, and 10. Plasma concentrations of zamicastat and its metabolites BIA 5-453 and BIA 5-961 were determined by SYNLAB Analytics & Services Switzerland AG (Sternenfeldstrasse 14 CH-4127 Birsfelden, Switzerland), in accordance with the applicable principles of GLP, using a previously validated LC-MS/MS analytical method.

The pharmacokinetics analysis set (PK population) included all subjects who had received at least one dose of zamicastat without major protocol deviation and had at least one valid evaluable post-dose plasma zamicastat concentration.

Safety and Tolerability Analysis

Safety parameters were carefully monitored throughout the study. Clinically significant abnormalities in physical examination, vital signs, 12-lead ECG, laboratory safety tests, and any other relevant safety variables were reported as adverse events (AEs), which were tabulated and summarized using MedDRA 23.1.

Results of vital signs, clinical laboratory variables, and ECG parameters [HR and the intervals: PR, QRS, QT, QT interval corrected with Bazett's formula (QTcB), QT interval corrected with Fridericia's formula (QTcF)], at each time point of measurement, were compared to baseline.

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The safety analysis set (safety population) included all subjects who received at least one dose of the investigational product.

Statistical Analysis

Estimation of parameters and statistical analyses on PK and PD data were conducted using non-compartmental analysis in Phoenix® WinNonlin® version 8.2 (Certara USA Inc, Princeton, NJ) and SAS® version 9.4 (SAS Institute, Cary, NC). For the additional statistical analyses, SAS® 9.4 was also used.

Descriptive statistics were calculated according to the variable nature: i) for continuous variables, number of subjects (n), arithmetic mean, standard deviation (SD), standard error of the mean and range (minimum and maximum); median, quartiles, coefficient of variation (CV%), geometric mean, geometric standard deviation, and geometric CV% are given, if pertinent; ii) for categorical variables, number of subjects, relative and absolute frequencies.

Actual p-values were reported, no multiplicity or adjustment of type I error was implemented; therefore, an alpha of 0.05 (p[?]0.05) was set for statistical significance. Hypotheses testing was defined to statistically compare the main primary PD endpoints of the study with zamicastat and placebo (SBP, DBP, and HR), with clinical margins of 5.5 mmHg, 3.7 mmHg and 4.1 bpm for mean SBP, DBP, and HR, respectively. Hence the null hypotheses were as follows:

SBP: ?0:??-??[?]-5.5

DBP: ?0:??-??[?]-3.7

HR: ?0:??-??[?]-4.1,

where ?? and ?? represent the mean of each endpoint for zamicastat and placebo, respectively.

Missing data were not replaced, and no imputation was conducted; descriptive statistics and statistical analysis were performed based on the available data only.

RESULTS

Study population

A total of 57 male subjects were screened for participation however, 22 subjects were randomized and participated in the study.

Out of the total, 35 participants were not admitted to randomisation due to the following reasons: 21 subjects did not meet one or more of the selection criteria (16 at screening and 5 at admission), 7 subjects decided to discontinue after the screening procedures, 3 subjects were excluded due to an AE, 1 subject was lost to follow-up, 1 subject was discontinued according to physician's decision, and 2 subjects served as back-up.

Regarding the 22 subjects admitted to randomisation, their age ranged between 20 and 53 years (mean +- SD: 34 +- 7.5 years) and the majority were Caucasian (81.8%). Weight varied between 57.8 kg and 93.4 kg (76.4+-10.1kg) and height was in the range of 167 cm and 190 cm (179+-6.2 cm); mean body mass index (BMI) 23.8+-2.8 kg/m^2). All these 22 subjects received at least one dose of IMP and had at least one valid post-dose PD assessment (Safety population = 22 subjects; PD population = 22 subjects). Of those, 21 subjects were included in the PK set, completed both study periods without major protocol deviations, and had at least one valid evaluable plasma zamicastat concentration (PP-PD population = 21 subjects; PK population = 21 subjects). A total of 21 subjects completed the study, as one subject had a serious adverse event and discontinued after first dosing, in period 1 of the study. This subject was excluded from the PP-PD and PK populations since he was only dosed with placebo. No concomitant medications administered to study subjects were considered to have a relevant impact on PK, PD, and safety analyses.

Cold pressure test

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The arithmetic means of SBP, DBP, and HR in each of the three phases of CPT, at day -1 and day 10, following administration of zamicastat and placebo are presented in **Table 1** . The CPT was effective in eliciting a BP and HR response. During the cold stimulus phase of the CPT, SBP, DBP, and HR increased significantly in relation to the rest phase, irrespective of study day (day -1 or day 10) and treatment (zamicastat or placebo) (**Table 2**).

In comparison to placebo, the difference between cold stimulus and rest phases at day 10 adjusted to baseline (day -1), following administration of zamicastat, showed an estimated decrease of -4.62 mmHg for SBP which attained statistical significance (p=0.020). For DBP and HR, the difference between zamicastat against placebo was -1.86 mmHg and 0.81 bpm respectively; however, they were not statistically significant (p>0.05) (**Table 3**).

Compared to placebo, the difference between cold stimulus and rest phases at day 10, adjusted to baseline (day -1) following administration of zamicastat showed a statistically significant decrease of -2.62 mmHg in MAP response to cold stimulus during CPT (p=0.025), but not for the other haemodynamic parameters assessed (**Table 4**). Maximum response achievable (E_{max}) and time to occurrence of E_{max} (TE_{max}) for SBP, DBP, and HR were similar between zamicastat and placebo treatments, and the area under the effect time curve (AUEC) was slightly decreased with zamicastat treatment when compared to placebo. However, there were no statistically significant differences between zamicastat and placebo in E_{max}, TE_{max} and AUEC derived for SBP, DBP, and HR, following 10 days of treatment.

At each CPT phase (rest, cold stimulus, and recovery), no differences between zamicastat and placebo adjusted to baseline were observed for SBP, DBP, HR, MAP and haemodynamic parameters (CO, CI, SV, SVI, TPR, dP/dT and LVET)-..

Plasma Catecholamines

At day 10, zamicastat elicited a significant increase of 12.63 ng/L (p=0.040) and 19.22 ng/L (p=0.001) in plasma DA before CPT and after CPT. For day -1 and 10, following treatment with zamicastat and placebo, no differences were observed for DA, EPI, and NE plasma concentrations, when adjusted to baseline plasma concentrations, after CPT and before CPT. After CPT, a significant difference between zamicastat and placebo of 15.93 ng/L was observed (p=0.040) on the change from baseline EPI plasma concentration. For DA and NE change from baseline plasma concentrations, no statistically significant differences (p>0.05) were observed (**Table 5**).

Twenty-four-hour urinary excretion profile of catecholamines and catecholamine metabolites
The observed 24-hour urinary excretion of DA, EPI, and NE was generally lower with zamicastat than with placebo from day 3 to day 10 (n=21). At day 10, zamicastat was shown to have a statistically significant decrease in EPI (-3.96 µg/24h, p=0.007) and NE (-7.54 µg/24h, p=0.017) on the change from baseline 24-hour urine excretion profile when compared to placebo. No difference was observed for DA 24-hour urinary excretion profile, following 10 days of treatment (**Figure 2**). Compared to placebo, zamicastat was associated to an increase in HVA urinary excretion of 1.75 mg/24 h (p<0.001) and a decrease in VMA urinary excretion of 1.97 mg/24 h (p<0.001), which was observed from baseline up to day 10 (**Figure 2**). No differences were observed for normetanephrine or metanephrine.

Δοπαμινε-β-Hψδροξψλασε (ΔβH) αςτιιτψ

Following multiple-dose administration of zamicastat 400 mg, the pre-dose plasmatic inhibition of DβH activity ranged from 19.8% on day 3 to 25.0% on day 10 (**Figure 3)** . The variability between individuals was moderate, with coefficients of variation ranging from 32.6% to 39.8% during the 10 days of evaluation. With placebo, the inhibition of DβH activity ranged from -3.9% (day 3) to -4.3% (day 10). Compared to placebo, zamicastat was associated with a statistically significant increase in the inhibition of plasma DβH activity (p<0.001) from day 3 up to day 10.

Pharmacokinetic results

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Geometric mean trough plasma concentration *versus* time profiles of zamicastat, following administration of zamicastat 400 mg once daily for 10 days, are displayed in **Figure 2** , in linear scale (n=21). From visual inspection, steady-state plasma concentrations of zamicastat and its metabolites appeared to be reached at day 6 approximately. The geometric mean of zamicastat concentration ranged from 45.86±1.46 ng/mL on day 3 to 58.64±1.52 ng/mL on day 10. The inter-individual variability was moderate, with coefficients of variation ranging from 32.6% to 36.6% during the 10 days of zamicastat 400 mg once daily administration.

Safety results

Sixteen subjects reported a total of 26 treatment-emergent adverse events (TEAEs). Among the 22 subjects who received placebo, 6 (27%) reported a total of 10 TEAEs; 9 were considered drug-related. Among the 21 subjects who received zamicastat, 12 (57%) reported a total of 16 TEAEs; of these, 13 were considered related to the treatment. The most common drug-related TEAE was "orthostatic hypotension" of mild intensity, which was reported by 4 (18%) subjects with placebo and 5 (24%) subjects with zamicastat. One TEAE ("acute cardiac event"), which occurred with placebo, was considered of severe intensity. It was considered a serious adverse event (SAE) due to hospitalization, leding to subject withdrawal from the study. All remaining TEAEs were of mild intensity. No deaths, further SAEs, or discontinuations due to TEAEs were registered, and no clinically relevant change or clinically significant abnormality in vital signs, physical examinations, and clinical laboratory tests were observed. Vital signs and ECG parameters did not show any overall trend.

DISCUSSION

Herein, we describe a single-centre, prospective, double-blind, randomised, placebo-controlled, two-treatment, two-sequence, two-period crossover study with 400 mg zamicastat/placebo, in healthy male subjects. The cold stimulus caused a marked sympathetic response evidenced by a significant increase in BP and HR during CPT, at baseline (day -1) and after a 10-day treatment with zamicastat or placebo.

CPT was selected to explore the effect of oral zamicastat, at steady-state conditions, on the overdrive of the sympathetic system and consequent effects on the cardiovascular system. This test has been used both clinically and experimentally to evaluate non-baroreflex-mediated sympathetic neural control in humans, cardiovascular reactivity to stress in normotensive and hypertensive subjects, and the efficacy of lifestyle and pharmacological interventions on BP and vascular reactivity.

Even though our results followed the tendencies presented in other reports, showing increased BP and HR, the increment in HR was lower than in other studies, in which the increase could be higher than 20 bpm, after a 90-second immersion in cold water. However, in other studies CPT showed to have lower or no effect on HR. On the other hand, the change in BP was higher than in previous studies, reaching about 30 mm Hg. These variations may be related to differences in the baseline BP values of the participants as well as to other demographic and behavioural patterns such as age, gender, weight, and exercise habits, which have been shown to influence reactivity to cold-induced stress. Despite the differences, our results on day 10 illustrate the absence of habituation or learning from exposure to the cold stimulus. In fact, both BP and HR kept the same increasing trend with similar magnitude, after 10 days. This observation combined with the referred influence of demographic and behavioural patterns on the reactivity to CPT can guide the definition of future selection criteria that can guarantee the inclusion of subjects with more robust baseline conditions to promote adaptation and learning during cold exposure. This would enrich the cohort by reducing selection bias. However, this endeavour requires a more detailed analysis of the correlation of the baseline characteristics of our participants with habituation to the cold stressor.

Women were not eligible for this study to avoid the potential influence of fluctuation of sex hormones throughout the menstrual cycle with neurohumoral variations with impact on the cardiovascular system.

Compared to placebo, zamicastat significantly decreased SBP and MAP response to cold stimulus during CPT, following 10 days of treatment, evidencing its effect on the overdrive sympathetic response to cold stimulus. Zamicastat reduced SBP (-4.62 mmHg) and DBP (-1.86 mmHg) when compared with placebo.

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The HR slightly increased (0.81 bpm) which may be related to compensatory hemodynamic effects. The one-sided 95% confidence interval (-7.77 mmHg) was below the established clinical margin for SBP (-5.5 mmHg) supported by a statistically significant difference (p=0.020) between zamicastat and placebo.

The measurement of plasma and urine catecholamines proved to be a valid tool to evaluate the impact of zamicastat on the SNS. These data showed that zamicastat 400 mg, given once daily, inhibited DβH plasmatic activity, increased the 24-h urinary excretion of HVA, and significantly decreased the 24-h urinary excretion of EPI, NE, and VMA, when compared to placebo. The decrease of NE in urine 24h explains the reduction of its metabolite VMA, which reflects a decrease in the total production of catecholamines caused by zamicastat treatment. On the other hand, the levels of DA in urine 24h were similar with zamicastat and placebo, but its metabolite HVA increased, which may imply that a higher amount of DA was metabolized. Our results confirm that the 24h urinary catecholamines is a collector of plasmatic levels of catecholamines and, as such, is more assertive as a potential biomarker of SNS modulation than the sporadic assessment of catecholamines in plasma, which is more affected by fluctuations and interferences than an integrated measurement over 24 h.

Overall, the profile of AEs showed that placebo and zamicastat treatments were similarly well tolerated.

Our results suggest that mechanism of action mediated by zamicastat can be a promising therapeutic option for morbidities characterized by excessive activation of the SNS, such as PAH, ischemic disease, among others.

In conclusion, our study confirmed the effect of zamicastat on the SNS through the decrease of SBP and MAP response to cold stimulus during CPT and inhibition of DβH plasmatic activity. In addition, the effect of zamicastat in 24 h urine catecholamines and their metabolites proved to be clinically relevant, thereby confirming its potential as SNS modulator.

Funding statement

This study was sponsored by Bial-Portela & C^{a} S.A.

Conflict of interest disclosure

CR, GCF, HG, and LM are or were employees of Bial-Portela & C^{a} S.A.

MF, SCH, FP, and LA are or were employees of BlueClinical.

NS is a Consultant of BlueClinical.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval statement

The study was implemented and conducted according to the principles of the Declaration of Helsinki and of the ICH Good Clinical Practice. The protocol (BIA-51058-123, EudraCT No 2020-003262-40) was approved by the National Ethics Committee for Clinical Research (CEIC).

Patient consent statement

Written informed consent was obtained from each prospective subject prior to any study procedure, after the physician in charge had the conviction that the subject was aware of the implications of participating in the study and the subject confirmed his willingness to participate. Subjects were assured that they might abandon the study at any time without any prejudice.

References

Table Captions

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Table 1 . Arithmetic means (SD) of SBP, DPP, and HR measured during CPT, at day -1 and day 10, following administration of zamicastat and placebo (PP-PD population, n=21).

Table 2. Arithmetic means (SD) of the differences between cold stimulus and rest phases for SBP, DPP, and HR measured during CPT, at day -1 and day 10, following administration of zamicastat and placebo (PP-PD population, n=21).

Table 3. Comparison of zamicastat against placebo for cold stimulus and rest phases (at day 10 adjusted to baseline) (PP-PD population, n=21).

Table 4. Mixed effect model results of mean arterial pressure and hemodynamic parameters during cold pressor test – Least square means differences between zamicastat and placebo for the difference between cold stimulus and rest phases adjusted to baseline (PP-PD population, day -1, n=21).

Table 5. Mixed effect model results of plasma catecholamines concentration – differences between zamicastat and placebo – Least square means before and after CPT at day 10, adjusted to baseline (PP-PD population, n=21).

Figure Captions

Figure 1. Study design diagram

Figure 2. Least square means and corresponding standard error of dopamine (A), epinephrine (B), norepinephrine (C), normetanephrine (D), homovanillic acid (E), and vanillylmandelic acid (F) 24-hour urine excretion profiles

Figure 3. Percentage (arithmetic mean) of plasmatic inhibition of DβH activity at pre-dose (trough) following administration of zamicastat 400 mg (once daily) and placebo (PP-PD population, n=21). (DβH: dopamine-β-hydroxylase; PP-PD: per-protocol pharmacodynamics)

Tables

Table 1. Arithmetic means (±SD) of SBP, DPP, and HR measured during CPT, at day -1 and day 10, following administration of zamicastat and placebo (PP-PD population, n=21).

	Treatment Period Day	**Treatment Period Day**	**Treatment Period Day**	**Treatment Period Day**	**Treatment Period Day**	**Treatment Period Day**
	Day -1 Rest	**Day -1 Cold Stimulus**	**Day -1 Recovery**	**Day 10 Rest**	**Day 10 Cold Stimulus**	**Day 10 Recovery**
Systolic Blood Pressure (mmHg)	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**
Zamicastat	119±9	149±15	125±10	124±11	156±15	130±11
Placebo	121±13	150±18	128±14	122±14	158±19	131±13
Diastolic Blood Pressure (mmHg)	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**
Zamicastat	59±6	78±10	63±7	61±4	82±10	65±7
Placebo	59±8	78±13	63±8	60±5	82±11	65±7
Heart Rate (bpm)	**Heart Rate (bpm)**	**Heart Rate (bpm)**	**Heart Rate (bpm)**	**Heart Rate (bpm)**	**Heart Rate (bpm)**	**Heart Rate (bpm)**
Zamicastat	63±8	70±9	59±6	63±9	73±8	60±9
Placebo	62±7	71±9	59±6	62±6	72±9	59±6

	Treatment Period Day	Treatment Period Day	Treatment Period Day	Treatment Period Day	Treatment Period Day	Treatment Period Day
CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmaco-dynamics; SBP: systolic blood pressure.

Table 2. Arithmetic means (±SD) of the differences between cold stimulus and rest phases for SBP, DPP, and HR measured during CPT, at day -1 and day 10, following administration of zamicastat and placebo (PP-PD population, n=21).

	Difference cold stimulus – rest Day -1	**Difference cold stimulus – rest Day 10**
Systolic Blood Pressure (mmHg)	**Systolic Blood Pressure (mmHg)**	**Systolic Blood Pressure (mmHg)**
Zamicastat	30±11	32±11
Placebo	29±10	36±9
Diastolic Blood Pressure (mmHg)	**Diastolic Blood Pressure (mmHg)**	**Diastolic Blood Pressure (mmHg)**
Zamicastat	19±6	21±7
Placebo	19±8	22±8
Heart Rate (bpm)	**Heart Rate (bpm)**	**Heart Rate (bpm)**
Zamicastat	8±6	9±7
Placebo	10±6	11±6
CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmacodynamics; SBP: systolic blood pressure; SD: standard deviation	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmacodynamics; SBP: systolic blood pressure; SD: standard deviation	CPT: cold pressure test; DPP: diastolic blood pressure; HR: heart rate; n: number of subjects; PP-PD: per-protocol pharmacodynamics; SBP: systolic blood pressure; SD: standard deviation

Table 3. Comparison of zamicastat against placebo for cold stimulus – rest phases (at day 10 adjusted to

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baseline) (PP-PD population, n = 21)

SBP (mmHg)					
DBP (mmHg)					
HR (bpm)					
Estimates correspond to the difference between zamicastat and placebo for cold stimulus – rest phases on the change from b					

Table 4. Mixed effect model results of mean arterial pressure and hemodynamic parameters during cold pressor test – Least square means differences between zamicastat and placebo for the difference between cold stimulus and rest phases adjusted to baseline (day -1, PP-PD population, n=21).

Mean Arterial Pressure (mmHg)					
Cardiac Output (L/min)					
Cardiac Index (L/min/m^2)					
Stroke Volume (mL)					
Stroke Volume Index (mL/m^2)					
Total Peripheral Resistance (mmHg.s/mL)					
Cardiac Contractility (mmHg/s)					
Left Ventricular Ejection Time (ms)					
Estimates correspond to the difference between zamicastat and placebo for cold stimulus – rest phases on the change from b					

Table 5 . Mixed effect model results of plasma catecholamines concentration – differences between zamicastat and placebo – Least square means before and after CPT at day 10 adjusted to baseline (PP-PD population, n = 21)

Variable	**Before CPT Estimate***	**Before CPT One-sided 95% CI**	**Before CPT p-value**	**After CPT Estimate***	**After CPT One-sided 95% CI**
Dopamine (ng/L)	20.70	-6.43	0.204	18.72	-5.67
Epinephrine (ng/L)	13.86	-0.93	0.122	15.93	3.30
Norepinephrine (ng/L)	-27.74	-102.86	0.541	-18.16	-93.28

*Estimates correspond to the difference between zamicastat and placebo on the change from baseline.

CI: confidence interval; CPT: cold pressure test; PP-PD: per-protocol pharmacodynamics.

Figures

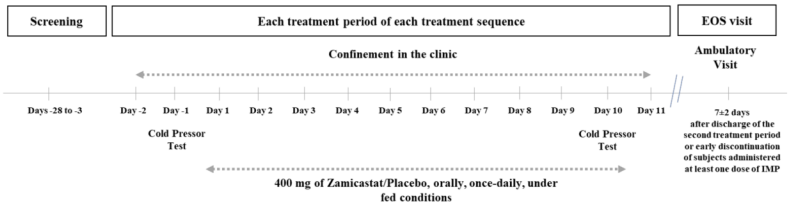

Figure 1. Study design diagram

(EOS: end of study)

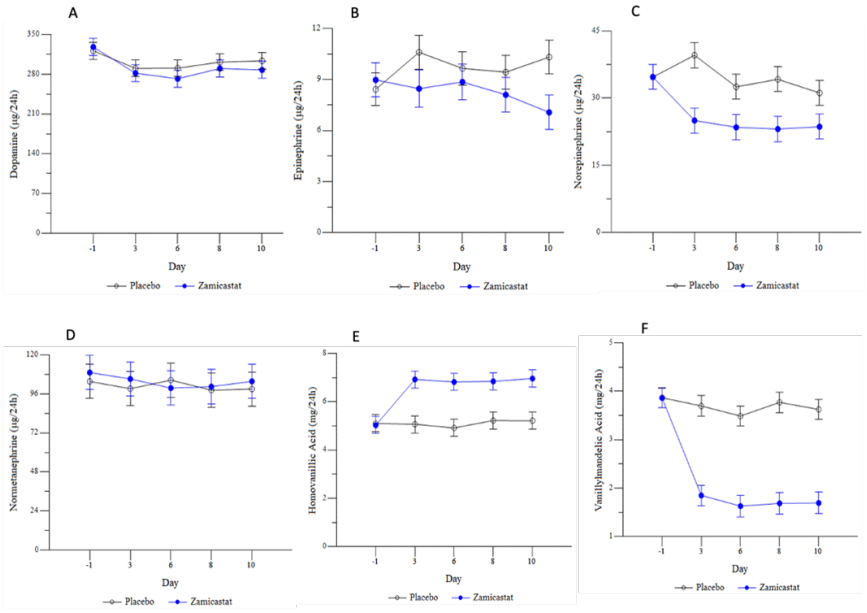

Figure 2. Least square means plots of dopamine (A), epinephrine (B), norepinephrine (C), normetanephrine (D), homovanillic acid (E), and vanillylmandelic acid (F) 24-hour urine excretion profiles.

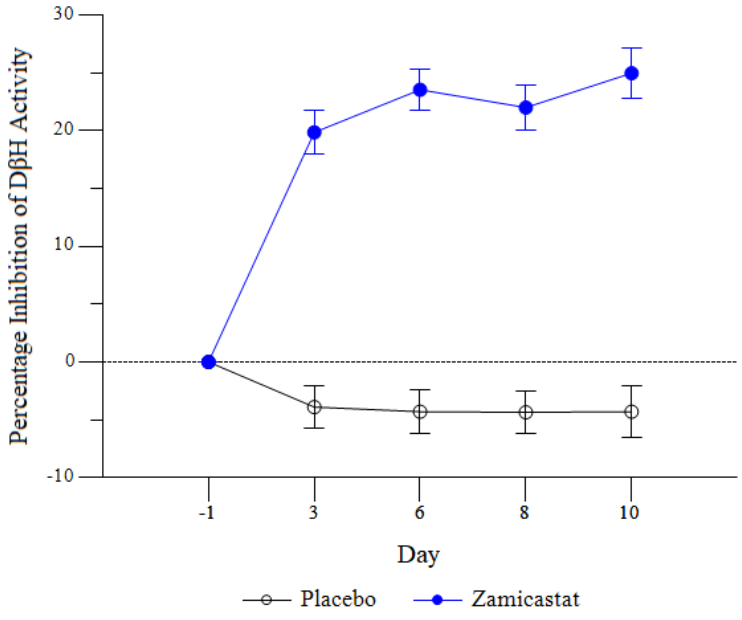

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Figure 3 . Percentage of plasmatic inhibition of DβH activity at pre-dose (trough) following administration of zamicastat 400 mg (once daily) and placebo (PP-PD population, n = 21).

(DβH: dopamine-β-hydroxylase; PP-PD: per-protocol pharmacodynamics).

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