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Safety, Tolerability, and Pharmacokinetics of RT234 (Vardenafil Inhalation Powder): A First-in-Human, Ascending Single- and Multiple-Dose Study in Healthy Subjects

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Abstract

Background: RT234 (vardenafil inhalation powder) is being developed for pulmonary administration ''as needed'', to acutely improve exercise tolerance and symptoms in patients with pulmonary arterial hypertension (PAH).
Methods: This single-center, open-label, randomized study in 32 healthy adult subjects evaluated single and multiple inhalation doses of RT234, for safety, tolerability, and pharmacokinetics (PKs).
Results: RT234 was generally safe and well tolerated at single doses of 0.2–2.4 mg and after repeated dose administration of up to 2.4 mg q4h for four doses daily for 9 days. The most common treatment-emergent adverse events were mild-to-moderate headaches. There was no evidence of pulmonary irritation or inflammation. Vardenafil was absorbed very rapidly after inhalation as RT234, independent of dose level and number of doses administered. The t_{max} occurred at the time that the first blood sample following completion of dosing. After C_{max} was achieved, plasma vardenafil concentrations declined rapidly in an exponential fashion that appeared to be parallel among dose levels. Vardenafil plasma concentrations and PK parameters increased in a dose-proportional manner. Vardenafil systemic exposure was notably greater after oral administration of 20 mg vardenafil tablets (Levitra®) than after administration of any dose level of RT234. During repeated dose administration of RT234, C_{max} was attained rapidly following each dose and in a pattern similar to that observed after single-dose administration. Minor accumulation, characterized by very low mean morning predose vardenafil concentrations (<0.5 ng/mL), occurred after q4h dosing of up to four doses per day for 9 days. Taken together, these findings show that no clinically important vardenafil accumulation is likely after repeated-dose administration of RT234. Mean vardenafil $t_{1/2}$ values were comparable after single- and repeated-dose administration.
Conclusions: Comparative plasma vardenafil bioavailability data from this study provide scientific justification for reliance on Food and Drug Administration findings for Levitra tablets. These findings support further evaluation of RT234 for as-needed treatment of patients with PAH. The Clinical Trials Registration number is ACTRN12618001077257.

Keywords: inhalation, pharmacokinetics, pulmonary arterial hypertension, vardenafil

Introduction

Pulmonary arterial hypertension (PAH) is characterized by progressive pulmonary vascular remodeling resulting in right ventricular dysfunction, increases in pulmonary artery pressure, pulmonary vascular resistance and right heart failure, and eventually death.[1]

The treatment of PAH has evolved from no approved therapies about 20 years ago, to more than a dozen therapies today.[2] Current multidrug regimens for PAH aim to

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alleviate vasoconstriction, vascular endothelial cell proliferation, smooth muscle cell proliferation, and endothelial dysfunction within pulmonary arteries.[3,4] Nonetheless, PAH remains a mortal disease with a median survival comparable to lung cancer (i.e., 7–8 years).[2]

Patients typically present with dyspnea, physical fatigue, and impaired exercise capacity. As the disease progresses, these symptoms increase, and dramatically impact day-to-day functioning and patient quality of life (QoL).[2,5] In 2014 Food and Drug Administration (FDA) held a public meeting to hear perspectives from patients on living with PAH, its impact on their daily life, and the impact of currently available therapies on their lives.[5] According to patients, the symptoms of PAH make it increasingly difficult to participate in household activities or to hold a job outside the home. Tasks that were once easy (e.g., climbing stairs, exercising, cleaning the house, shopping, or even playing with their children), become increasingly difficult and often leave them breathless. In an ideal situation, current maintenance therapies would enable patients to perform desired activities, unfortunately, it is clear that this is not always the case.

As such, there remains a significant unmet need for treatments that acutely reduce symptoms and improve exercise tolerance, enabling patients to function more normally in their day-to-day lives.

RT234 (vardenafil inhalation powder) is a dry powder formulation of vardenafil hydrochloride. RT234 is not intended to be used as a maintenance therapy, but instead is to be used ''as-needed'' (i.e., pro re nata [PRN]), to enable short-term symptom control and improvements in the ability to perform activities of daily living. Over time, it is hoped that enabling patients to be able to do more activities will result in improvements in their QoL.

The proposed PRN use of RT234 is analogous to how short-acting β-agonists (SABA) are used in asthma treatment. SABAs are used in addition to inhaled corticosteroid/long-acting β-agonist maintenance therapies, to provide acute improvements in breathlessness during episodes of acute wheeze, and bronchodilation before exercise for patients with exercise-induced bronchospasm.

Critical design attributes for an as-needed dosage form include: a rapid onset of action, a suitable duration of action to complete a targeted activity, minimal safety and tolerability issues when used in addition to maintenance therapies, and a simple, noninvasive, portable delivery system with low daily treatment burden.

In this article, we report the results of Study RT234-CL101, a Phase 1, first in human, safety, tolerability, and pharmacokinetic (PK) study of RT234. The study comprises single and multiple ascending dose portions, plus a crossover component in the single-dose portion to estimate the bioavailability of vardenafil following inhalation of RT234 relative to a 20 mg oral vardenafil tablet (Levitra®; Bayer Healthcare AG, Leverkusen, Germany).

Materials and Methods

Materials

Oral tablets comprising 20 mg of vardenafil (Levitra, Bayer Healthcare AG) were obtained from the local pharmacy. Levitra represents the Reference Listed Drug for a 505(b)(2) regulatory submission with FDA. The results presented herein are intended to provide a scientific bridge to utilization of the Agency's previous findings of safety and effectiveness for vardenafil.

The RT234 drug product was manufactured for Respira by Hovione FarmaCiencia (Loures, Portugal). Fine micronized vardenafil hydrochloride (Zaklady Farmaceutyczne Polpharma S.A., Gdansk, Poland) and coarse lactose monohydrate particles (DFE Pharma, Beltsville, MD) were combined with a high shear mixer to yield an adhesive mixture of drug and carrier. The resulting ''lactose blend'' was filled into size 3 hypromellose capsules (VCaps®; Capsugel, Greenwood, SC) with a dosator-based filling process. Filled capsules were packaged in 40 cm^3 high-density polyethylene bottles (35 capsules/bottle). The bottles were then overwrapped in a laminated aluminum pouch and stored between 2°C and 8°C until use.

RT234 was administered through oral inhalation with an RS01 dry powder inhaler (DPI) (Plastiape S.p.A., Osnago, Italy). The RS01 DPI is a capsule-based, unit-dose, breath-actuated DPI that is fully mechanical, with a medium resistance to airflow ($R = 0.10$ cm $H_2O^{0.5}$ L^{-1} minute). In the context of this study all dose preparation steps (i.e., insertion of the capsule into the device and piercing of the capsule) were done by the healthcare professional (HCP) assisting in the dosing. The patients were instructed in proper dose inhalation at each dosing event (i.e., inhale with maximal effort until your lungs are full). Two inhalations were performed on each capsule to ensure that the dose was delivered. This was subsequently confirmed by inspection of the empty capsules following dose administration. The nominal dose of vardenafil in RT234-CL101 ranged from 0.2 to 9.6 mg/day. The dose range was achieved with a single powder formulation (2.0% w/w vardenafil) by varying the capsule fill mass (10 mg, 30 mg), the number of capsules administered (1, 2, and 4), and the number of doses administered (up to four times/day) (Table 1).

The doses of RT234 were selected based on *in vitro* aerosol testing in a Next-Generation Impactor using the fine particle fraction <5 μm (i.e., $FPF_{<5\mu m}$) as a surrogate for the total lung dose. The $FPF_{<5\mu m}$ of RT234 at a 4 kPa pressure drop was $\sim$30% of the nominal dose. Given that vardenafil is a lipophilic drug that is not dissolution limited, we anticipate that systemic absorption of drug deposited within the lungs will approach 100%. Hence, the pulmonary bioavailability can be approximated by the $FPD_{<5\mu m}$ ($\sim$30%). The oral bioavailability of vardenafil is $\sim$15%. Hence, the 2.4 mg dose of RT234 is anticipated to have a systemic exposure comparable to the 5 mg oral tablet, while administering the 2.4 mg dose four times daily is anticipated to have a daily systemic exposure roughly comparable to the 20 mg oral tablet.

Table 1. Doses Utilized in RT234-CL101

Study	*Nominal dose (mg/day)*	*Fill mass (mg)*	*No. capsules*	*No. doses/day*
SAD	0.2	10	1	1
SAD	0.6	30	1	1
SAD	1.2	30	2	1
SAD	2.4	30	4	1
MAD	4.8	30	4	2
MAD	7.2	30	4	3
MAD	9.6	30	4	4

SAD, single ascending dose; MAD, multiple ascending dose.

Study design and subjects

Study RT234-CL101 was a single-center, open-label, randomized study in healthy adult subjects consisting of an ascending single dose study (Part 1) followed by a repeated dose study (Part 2). The study was designed to evaluate safety and tolerability, and to characterize the PK profiles of single and repeated doses of RT234. In addition, vardenafil exposure after inhalation administration of a single 0.6 mg dose of RT234 was compared with that following oral administration of a single 20 mg Levitra tablet in a crossover fashion.

Planned enrollment was 32 subjects, with 6 subjects in each of four ascending single-dose cohorts in Part 1, and 8 subjects in Part 2. Subjects were screened from 28 days before randomization/dose administration and were admitted to the study clinic on Day 1 for review of inclusion and exclusion criteria before the start of study procedures on Day 1. Eligible subjects were healthy men and women (nonpregnant and nonlactating) 18–45 years of age. Figure 1a displays a schematic for the conduct of study Part 1.

Following completion of Part 1 of the study, the eight additonal subjects enrolled in Part 2 received the highest well-tolerated single dose of RT234 from Part 1 (i.e., 2.4 mg), administered every 4 hours (q4h) beginning with two doses on Day 1, 3 doses on Day 2, and 4 doses on Days 3 through 9. Figure 1b provides a schematic of the study design for Part 2. Enrolled subjects confined to the study clinic from Day 1 through 9, were discharged on Day 10 following completion of all study procedures, and returned for a follow-up visit on Day 14.

The trial was conducted in accordance with the ethical principles of Good Clinical Practice, according to the International Conference on Harmonization (ICH) Harmonized Tripartite Guideline (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (2000), using a protocol reviewed and approved by The Alfred Hospital Ethics Committee. The study was registered on the Australian New Zealand Clinical Trials Registry under the identifier: ACTRN12618001077257, and conducted at Nucleus Network (Melbourne, Australia).

Criteria for safety evaluation: safety

The safety and tolerability of single and repeated doses of RT234 and a single 20 mg oral dose of Levitra were investigated according to the following specific assessments: vital signs (systolic and diastolic blood pressure, heart rate, oral temperature, and respiratory rate), 12-lead electrocardiogram (ECG), spirometry [forced expiratory volume at 1 second

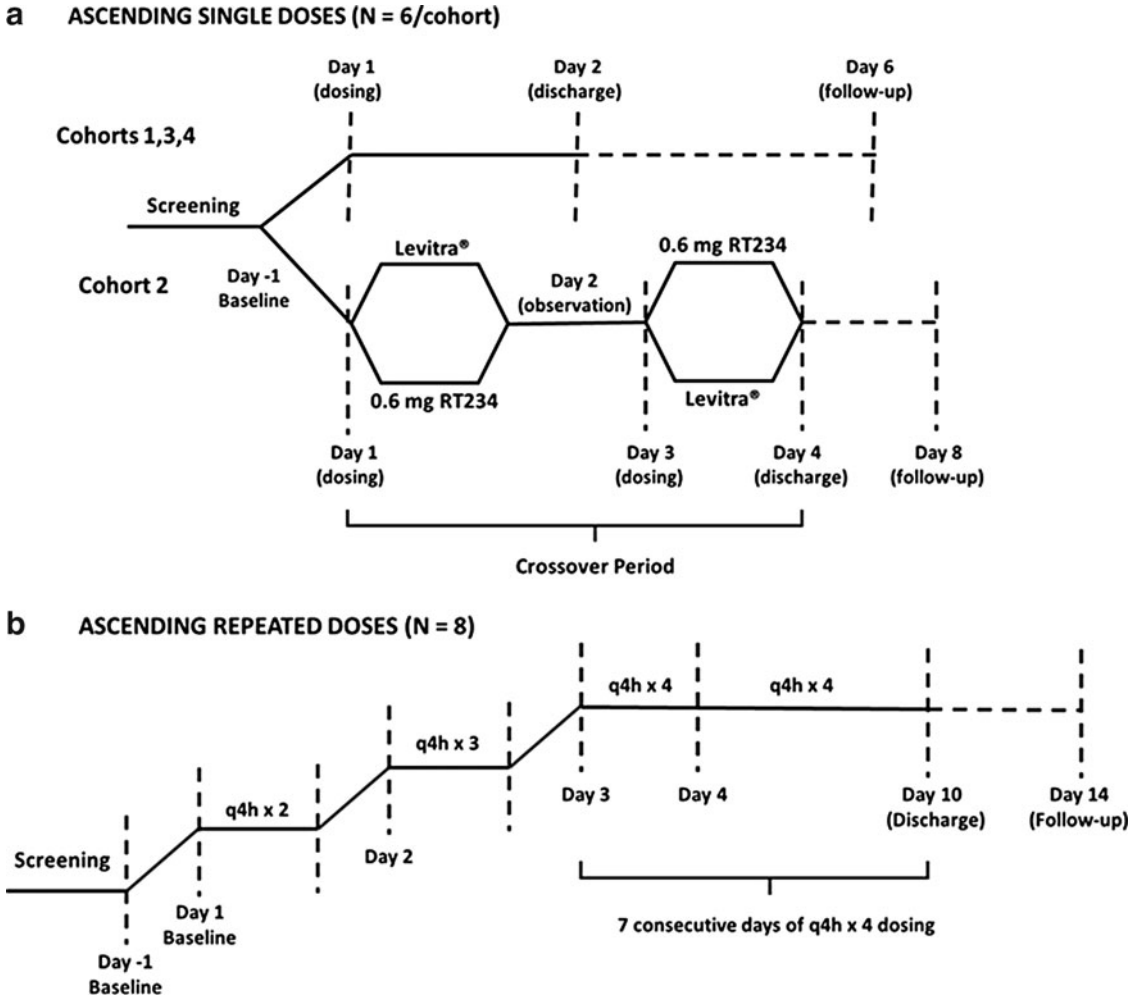

FIG. 1. Study design. **(a)** Part 1: SAD, single ascending doses; **(b)** Part 2: MAD, multiple ascending doses.

(FEV_1); forced vital capacity (FVC)], clinical laboratory tests (hematology, biochemistry, coagulation, and urinalysis), physical examination, and assessment of treatment-emergent adverse events (TEAEs).

Elicitation of AEs and serious adverse events (SAEs) occurred at each interaction with the subject, and AEs were recorded from the time of informed consent until the end of the post-treatment follow-up. The information on each AE or SAE included duration (start and stop time and date), severity, action taken, outcome, and assessment by the principal investigator of causality.

The study was subject to oversight by a Safety Monitoring Committee (SMC) comprising the Principal Investigator, the Respira Medical Officer/Designee, and a local medical monitor. Decisions to escalate the dose for cohort progression in Part 1 was dependent upon SMC review of safety and tolerability of subjects in the cohort.

The safety analysis dataset was defined as all eligible subjects who met inclusion criteria and received any amount of vardenafil as either RT234 or Levitra. AEs were coded using MedDRA Version 21.0. Incidence of AEs, severity, relationship to treatment, outcome, and actions taken were presented for each subject. In addition, listings of AEs leading to discontinuation of the study, SAEs, and deaths were provided as applicable. TEAEs were listed and summarized per treatment. The frequency of each AE was summarized by severity and relatedness to the investigational product. TEAEs were also summarized with frequency counts by system organ class (i.e., body system) and preferred term for each treatment dose group (Supplementary Data Section 4.0).

Subject disposition, demographics and baseline characteristics, medical history, prior and concomitant medications, study drug compliance, clinical laboratory results, vital signs, and ECG parameters were summarized and/or listed, as appropriate, according to the study statistical analysis plan.

Assessment of vardenafil PKs

Blood samples for PK analysis were obtained following administration of each single dose. Serial venous blood samples were to be collected within 15 minutes before dosing and between 2 minutes and 14 hours after initiation of dosing (16 time points).

During administration of repeated doses, serial blood samples were to be collected for day 1/dose 1, day 1/dose 2, day 2/dose 3, and day 9/dose 4. Trough samples were to be collected before the first dose on each day. Plasma concentrations of vardenafil at the various time points were determined with a validated liquid chromatography/mass spectrometry/mass spectrometry method (Supplementary Data, Section 1.1).

PK analysis population

The PK analysis population was defined as all subjects who received at least one dose of RT234 or the positive control Levitra, and had sufficient PK data to permit a meaningful PK analysis.

Noncompartmental PK analysis of single-dose data

After administration of single RT234 and Levitra doses in Part 1, noncompartmental methods were used to calculate vardenafil PK parameters, including the maximum plasma concentration (C_{max}), the time to the maximum concentration (t_{max}); the area under the plasma vardenafil concentration versus time curve (area under the curve [AUC]); and the apparent elimination half-life ($t_{1/2}$) using WinNonlin software (Pharsight Corporation, Mountain View, CA).

In Part 2 of the study, concentration versus time profiles from all subjects were simultaneously analyzed using nonlinear mixed-effects modeling performed using Monolix Version 3.2.1 software (Lixoft, Antony, France).[6]

Based on visual inspection of vardenafil concentration–time profiles after administration of RT234 and preliminary PK modeling, a two-compartment open PK model with first-order elimination and zero-order input was selected. Zero-order input was selected to reflect the study methods, where a single dosing occasion consisted of multiple inhalation doses administered serially at approximately evenly spaced time intervals (e.g., single doses in cohorts 3 and 4, and all repeated doses). Because subjects tend to inhale at different inspiratory flow rates and on different occassions, the input function was characterized as the total amount of drug inhaled divided by the total amount of time required to complete inhalation of the assigned number of capsules. The model was parameterized with fixed effects for apparent central volume (V/F), clearance (CL/F), peripheral compartment volume (Vz/F), and intercompartmental clearance (Q/F).[7] All parameters were treated as log-normally distributed during fitting. Details of model building and validation are described in Supplementary Data (Section 2.2).

Statistical considerations

No formal statistical testing was planned for this descriptive Phase 1 study. A sample size of six subjects per single-dose treatment group was deemed sufficient to meet the objectives of the study.

Results

Number of subjects planned and enrolled

Eighty-nine subjects were screened to enroll the planned 32 subjects (Part 1: 24 subjects, Part 2: 8 subjects). Twenty-two subjects completed Part 1 and five completed Part 2 per protocol, with two and three subjects, respectively, discontinued from dosing earlier than planned. Table 2 provides baseline demographics for the enrolled subjects.

Safety and tolerability

RT234 was well tolerated when administered to healthy male and female subjects at single inhaled doses of 0.2, 0.6, 1.2, and 2.4 mg; and at 2.4 mg doses administered two times every 4 hours (q4h) (Day 1), three times q4h (Day 2), and four times q4h (Days 3 through 9). These safety conclusions were based on the following findings from all 32 treated participants:There were no SAEs, life-threatening TEAEs, or TEAEs leading to death. The majority of TEAEs were mild, regardless of treatment or relatedness to study drug, and there were no severe TEAEs reported.

At least one TEAE was reported in 73.9% of subjects and 87.5% of subjects in Part 1 (SAD) and Part 2 (MAD), respectively. The incidence of TEAEs did not increase with increasing dose level. Treatment-related TEAEs were reported by a similar proportion of subjects in Part 1 (60.9%) and Part 2 (62.5%). The incidence of treatment-related

TABLE 2. BASELINE DEMOGRAPHICS—ASCENDING SINGLE DOSES (SAFETY POPULATION)

			RT234 0.6 mg (N = 6)				
Category	*Statistics*	*RT234 0.2 mg (N = 6)*	*RT234/oral vardenafil (N = 3)*	*Oral vardenafil/RT234 (N = 3)*	*RT234 1.2 mg (N = 6)*	*RT234 1.2 mg (N = 6)*	*Repeated-dose RT234 2.4 mg (N = 8)*
Age (years)	*n*	6	3	3	6	6	8
	Mean	23.7	23.0	27.3	24.8	26.8	27.1
	SD	3.78	4.36	4.93	7.36	6.27	2.42
	Median	23.5	25.0	25.0	23.0	27.5	27.5
	Min	19	18	24	18	18	24
	Max	30	26	33	38	36	31
Gender, *n* (%)	Male	1 (16.7)	1 (33.3)	1 (33.3)	1 (16.7)	4 (66.7)	7 (87.5)
	Female	5 (83.3)	2 (66.7)	2 (66.7)	5 (83.3)	2 (33.3)	1 (12.5)
Race, *n* (%)	Asian	1 (16.7)	1 (33.3)	0	2 (33.3)	2 (33.3)	1 (12.5)
	White	5 (83.3)	2 (66.7)	3 (100)	4 (66.7)	3 (50.0)	7 (87.5)
	Multiple	0	0	0	0	1 (16.7)	0
Ethnicity, *n* (%)	Hispanic or Latino	1 (16.7)	0	1 (33.3)	0	3 (50.0)	0
	Not Hispanic or Latino	5 (83.3)	3 (100)	2 (66.7)	6 (100%)	3 (50.0)	8 (100)
Body weight (kg)	*n*	6	3	3	6	6	8
	Mean	65.75	68.7	75.10	66.78	69.92	74.9
	SD	7.872	11.640	12.474	9.411	8.724	12.319
	Median	68.00	74.50	79.60	65.45	70.65	74.35
	Min	51.7	55.3	61.0	55.1	59.5	57.3
	Max	73.3	76.3	84.7	83.0	78.5	96.0
BMI (kg/m^2)	*n*	6	3	3	6	6	8
	Mean	22.43	23.57	23.83	23.55	23.87	24.46
	SD	2.545	2.597	1.656	2.824	2.726	3.176
	Median	22.35	23.00	24.0	23.60	24.90	25.45
	Min	19.7	21.3	22.1	20.5	20.3	20.2
	Max	26.4	26.4	25.4	28.1	26.6	28.6

Age (years) = integer value ([Date Signed Informed Consent − Date of Birth +1]/365.25).
BMI, body mass index; Max, maximum; Min, minimum; SD, standard deviation.

TEAEs did not increase with increasing dose level. Five (15.6%) participants had study drug withdrawn due to TEAEs, four of which had events that were considered related to treatment.

TEAEs are displayed in Table 3. Data from various cohorts in the SAD, MAD, and crossover portions of the SAD study, can be found in the Supplementary Data (Section 4.0). The most commonly reported TEAE was headache, regardless of dose or relationship to treatment.

Brief, asymptomatic decreases in systolic, diastolic, and mean arterial pressure were noted at ~40 to 100 minutes postdosing with RT234 and ~60 to 90 minutes postdosing with 20 mg oral vardenafil. These changes were not associated with reflex tachycardia or AEs of dizziness and were not considered clinically significant. One subject in Part 2 (MAD) experienced clinically significant changes in alanine aminotransferase and aspartate aminotransferase associated with an AE of mild viral hepatitis (acute cytomegalovirus titers positive), which was considered unrelated to study treatment. There were no other clinically significant laboratory values or study drug-related changes from baseline observed during the study. There were no clinically significant values or study drug-related changes in spirometry results, particularly FEV_1 or FVC, ECG parameters, or physical examination findings during the study.

Pharmacokinetics

The purpose of PK assessment in the single-dose portion of Study RT234-CL101 was to monitor plasma vardenafil exposure during assessment of safety and tolerability, and to characterize the noncompartmental PK of plasma vardenafil over a wide range of inhaled single doses.

Figure 2 shows that RT234 is very rapidly absorbed following oral inhalation at all dose levels, characteristic of pulmonary administration of lipophilic small-molecule drugs.[8] Vardenafil t_{max} typically occurred at the first sampling time after dosing (2 minutes) and was similar for the 0.2- and 0.6-mg dose levels (0.03 h = 2 min), when single capsules were inhaled to deliver the planned doses. The t_{max} occurred progressively later for the 1.2- and 2.4-mg dose levels, which required sequential administration of two and four capsules, respectively. For these dose levels, C_{max} was attained in ~4 and 10 minutes after the completion of dosing.

After C_{max} was achieved, plasma vardenafil concentrations declined rapidly in a multiexponential fashion that appeared to be parallel across dose levels, with drug detectable in plasma until ~4, 8, 14, and 14 hours postdose after administration of single 0.2-, 0.6-, and 1.2- and 2.4-mg doses, respectively. At the 0.2-mg dose level, the terminal elimination phase could not be determined because most

Table 3. Treatment-Related Treatment-Emergent Adverse Events for RT234 and Twenty Milligrams Oral Vardenafil in Healthy Volunteers

System organ class (preferred term)	*RT234 (N = 31)* N *(%)*	*Oral vardenafil (N = 5)* N *(%)*
Subjects with at least one TEAE	19 (61.3)	4 (80.0)
Nervous system disorders	12 (38.7)	4 (80.0)
Headache	10 (32.3)	4 (80.0)
Dizziness	4 (12.9)	4 (80.0)
Postural dizziness	1 (3.2)	0 (0.0)
Autonomic nervous system imbalance	1 (3.2)	1 (20.0)
Lethargy	1 (3.2)	0 (0.0)
Head discomfort	1 (3.2)	0 (0.0)
Eye disorders	2 (6.4)	2 (40.0)
Eye pain	1 (3.2)	1 (20.0)
Photophobia	2 (6.4)	0 (0.0)
Scleral hyperemia	0 (0.0)	1 (20.0)
Vascular disorders	1 (3.2)	1 (20.0)
Flushing	1 (3.2)	1 (20.0)
Gastrointestinal disorders	5 (9.7)	0 (0.0)
Nausea	3 (9.7)	0 (0.0)
Abdominal pain	2 (6.4)	0 (0.0)
Vomiting	1 (3.2)	0 (0.0)
Respiratory, thoracic, and mediastinal disorders	5 (16.1)	0 (0.0)
Cough	3 (9.7)	0 (0.0)
Nasal congestion	2 (6.4)	0 (0.0)
General disorders	1 (3.2)	1 (20.0)
Chills	1 (3.2)	1 (20.0)
Infections and infestations	1 (3.2)	0 (0.0)
URT infection	1 (3.2)	0 (0.0)
Reproductive system and breast disorders	1 (3.2)	0 (0.0)
Spontaneous penile erection	1 (3.2)	0 (0.0)

TEAE, treatment-emergent adverse event; URT, upper respiratory tract.

samples obtained after 4 hours postdose were below the limit of quantification, due to a combination of low dose level and bioanalytical sensitivity. Consequently, plasma vardenafil PK parameters $t_{1/2}$, $AUC_{(0-inf)}$, CL/*F*, Vz/*F*, and K_{el} were not calculated at this dose level. All subjects in the 0.6, 1.2, and 2.4 mg dosing cohorts met defined criteria for evaluation and all parameters were estimated.

Because of the missing $t_{1/2}$ values at the 0.2-mg dose level and the fact that the time of the last detectable concentration was different among the dose levels, $t_{1/2}$ and the parameters that require it for calculation [$AUC_{(0-inf)}$, CL/*F*, Vz/*F*] and $AUC_{(0-last)}$ could not be directly compared between dose levels. Plasma vardenafil PK parameters after single-dose administration of RT234 are presented in Table 4.

In general, mean plasma vardenafil C_{max} and AUC values increased with increasing dose, consistent with the mean plasma vardenafil concentration–time curves shown in Figure 2. Mean values for vardenafil $t_{1/2}$, CL/*F*, and Vz/*F* at the 0.6-mg RT234 dose level appeared to be less than those at the 1.2- and 2.4-mg dose levels, suggesting some departure from dose proportionality, but this pattern may be due to underestimation of $t_{1/2}$ at the 0.6-mg dose level secondary to detection of plasma concentrations for only 8 hours after dosing compared with 14 hours after dosing at the higher dose levels. Mean $t_{1/2}$, CL/*F*, and Vz/*F* values were quite similar at the 1.2- and 2.4-mg dose levels.

In addition to the comparisons of PK parameter values described above, power analyses of C_{max}, $AUC_{(0-last)}$ and $AUC_{(0-inf)}$ versus dose were used to assess dose proportionality.[9] Figure 3 shows individual-subject C_{max}, $AUC_{(0-last)}$ and $AUC_{(0-inf)}$ values as a function of RT234 dose, with each parameter increasing in an apparent linear fashion.

Comparison of inhaled and oral vardenafil PKs

Vardenafil was rapidly absorbed after oral administration of Levitra tablets, with C_{max} occurring $\sim$1 hour after dosing, followed by a rapid exponential decline in plasma vardenafil concentrations that was detectable in all subjects through the 14-hour sampling time (Fig. 4). The mean plasma vardenafil concentration–time profile after oral administration of Levitra tablets in this study was consistent with the known profile of Levitra tablets.[10]

Given the approximate 33-fold difference in dose, plasma vardenafil concentrations were orders of magnitude greater after oral Levitra compared with single dose administration of 0.6 mg RT234. However, vardenafil clearance was similar between products as evidenced by the parallel PK profiles when plotted on a log scale.

Plasma vardenafil C_{max}, t_{max}, and both AUC values reflect both the differences in rates of absorption and magnitude of systemic exposure evident in Figure 4, with RT234/oral Levitra parameter ratios illustrating the greatly reduced vardenafil exposure observed after the administration of 0.6 mg RT234 (Table 5, upper panel).

A parallel group comparison of exposure parameters and corresponding RT234/oral Levitra parameter ratios using data from the highest RT234 dose studied are also presented in Table 5 (bottom panel).

Comparison of RT234/oral vardenafil C_{max} and AUC ratios showed that C_{max} was 6.7-fold less and $AUC_{(0-inf)}$ was 12.5-fold less at the 0.6-mg RT234 dose level than the corresponding parameters for 20 mg Levitra tablets (Table 5). At the highest studied RT234 dose level of 2.4 mg, vardenafil C_{max} was 2.5-fold less and $AUC_{(0-inf)}$ was 2.9-fold less than those for 20 mg Levitra. These results demonstrate that vardenafil systemic exposure was notably greater after oral administration of 20 mg tablets than after administration of RT234 at either the 0.6- or 2.4-mg dose level.

On a dose-normalized basis, the $AUC_{(0-inf)}$ of the 0.6 and 2.4 mg RT234 doses were 2.7 and 2.8-fold higher than the oral drug product. This reflects the differences in relative bioavailability between the inhaled and oral drug products. The dose-normalized C_{max} values were 5.3 and 3.6-fold greater for the 0.6 and 2.4 mg RT234 doses, respectively. The increased normalized C_{max} values also reflect the more rapid absorption of inhaled vardenafil.

The results in Table 5 also allowed a direct comparison of intersubject variability in the form of coefficient of variation (CV%) values between the two routes of vardenafil administration.

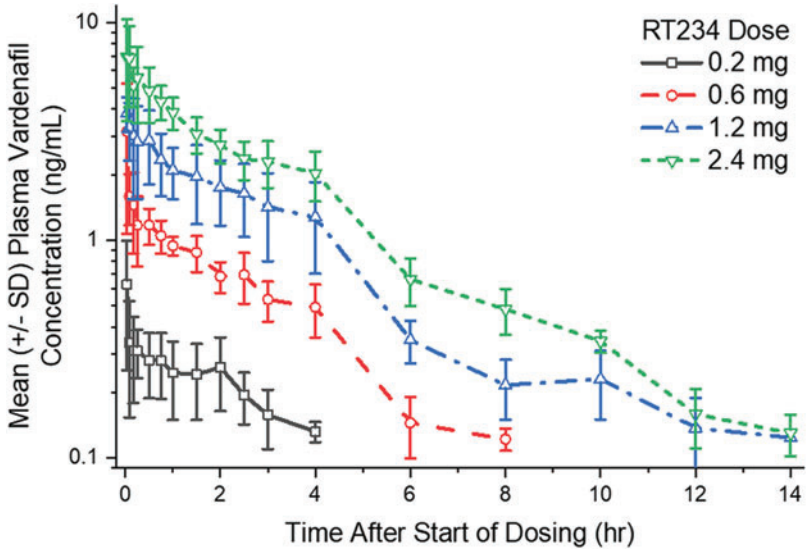

FIG. 2. Mean plasma vardenafil concentrations versus nominal time after administration of single ascending inhalation doses to healthy subjects. Color images are available online.

CV% values for $AUC_{(0–last)}$ and $AUC_{(0–inf)}$ after administration of RT234 ranged from 14 to 18 across the dose levels presented in Table 5, which was approximately half of the CV% observed after Levitra administration, and even less than previously reported (i.e., ~50%–60%).[10] The lower intersubject variability observed with RT234 is likely due to the lack of first-pass metabolism after pulmonary administration of RT234 as compared with the extensive hepatic first-pass metabolism reported after oral administration of vardenafil. Intersubject CV% values for C_{max} after administration of RT234 ranged from 40% to 65%, consistent with values reported for oral vardenafil.

Multiple ascending daily dose PK results (part 2)

The purpose of the MAD portion of Study RT234-CL101 was to monitor plasma vardenafil exposure during assessment of safety and tolerability of RT234 after administration of repeated ascending daily doses and to characterize the PKs of plasma vardenafil after repeated daily doses of up to 2.4 mg q4h for four sequential doses. The need to explore safety and tolerability at a clinically relevant dosing interval of q4h limited to waking hours did not result in attainment of PK steady state, and this limited the ability to capture and compare PK data that could be analyzed using noncompartmental methods.

A scatter plot of plasma vardenafil concentrations versus time pooled among all subjects is provided in Figure 5. This figure also shows when samples were collected relative to times of dose administration.

Individual subject concentration–time profiles after administration of Dose 2 on Day 1, Dose 3 on Day 2, and Dose 4 on Day 9 were similar within the period of repeated dosing and in comparison with the concentration–time profile after administration of a single 2.4 mg dose of RT234 during the SAD portion of the study (Fig. 2).

The same rapid attainment of C_{max} almost immediately after dosing was observed throughout the duration of RT234 multiple dose administrations (Fig. 6), consistent with results following single-dose administration. The rapid absorption was followed by comparable multiexponential disposition on Days 1, 2, and 9 when full sampling was performed. No clinically important accumulation of vardenafil was apparent on visual comparison of the fully sampled profiles after q4h dosing of up to four doses per day for 9 days; minor accumulation was visible in the morning

TABLE 4. PLASMA VARDENAFIL PHARMACOKINETIC PARAMETERS AFTER ADMINISTRATION OF SINGLE ASCENDING DOSES OF RT234

RT234 treatment	t_{max}[a] *(h)*	C_{max} *(ng/mL)*	$AUC_{(0–last)}$ *(h · ng/mL)*	$AUC_{(0–inf)}$ *(h · ng/mL)*	$t_{1/2}$ *(h)*	*CL/F (L/h)*	*Vz/F (L)*
0.2 mg (*N*=6)	0.03	0.6385 (56)	0.7838 (48)	ND	ND	ND	ND
0.6 mg (*N*=5)	0.03	3.178 (65)	4.052 (18)	4.348 (18)	1.749 (26)	142 (20)	348.9 (19)
1.2 mg (*N*=6)	0.06	4.302 (18)	10.52 (31)	11.03 (29)	2.678 (39)	116.8 (28)	438.1 (37)
2.4 mg (*N*=6)	0.12	8.467 (40)	18.20 (15)	18.67 (14)	2.483 (23)	130.7 (14)	469.2 (27)

Mean (CV%) presented unless otherwise indicated.
[a]Median t_{max} displayed.
CV%, coefficient of variation; inf, infinity; ND, not determined due to inadequate data available in the elimination phase to permit calculation of $t_{1/2}$; AUC, area under the curve.

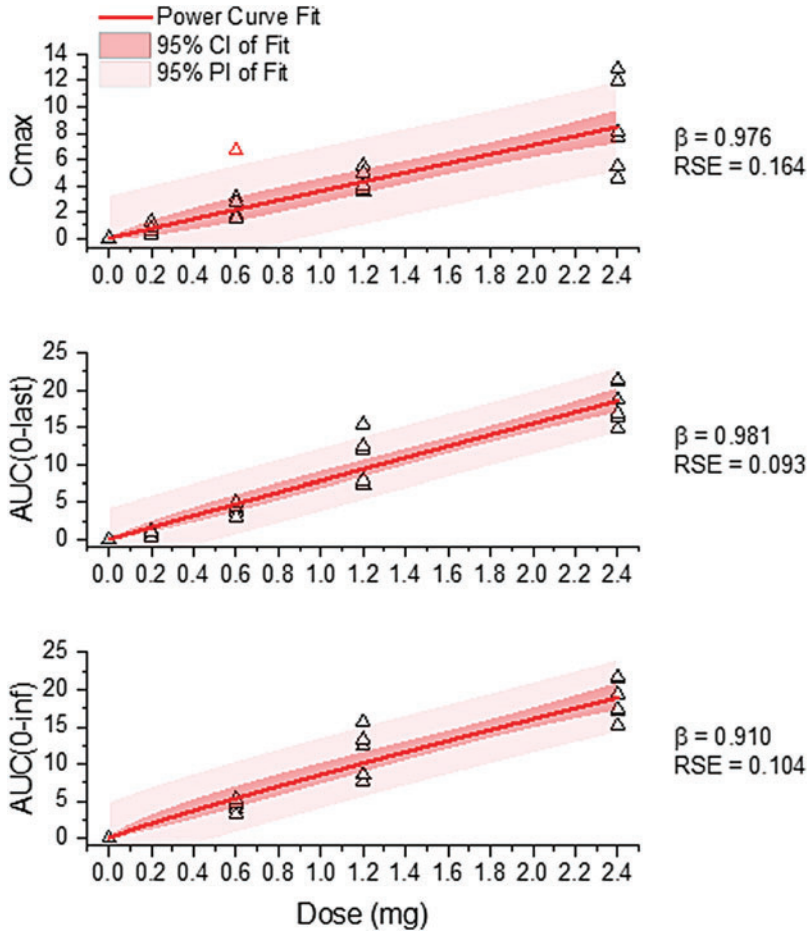

FIG. 3. Plasma vardenafil C_{max} and AUC values versus RT234 dose. AUC, area under the curve. Color images are available online.

predose samples on Days 4 through 9 with very low mean vardenafil concentration values (0.26–0.35 ng/mL), and no evidence of further accumulation after Day 4. Individual-subject plasma vardenafil concentrations vs. time are presented graphically in Supplementary Data (Section 3.1).

Summary C_{max}, t_{max}, and AUC values were comparable among days and administered doses (Table 6). Mean $AUC_{(0-4)}$ values for Doses 1 and 2 on Day 1 were also similar, confirming the assessment of similarity made from visual inspection of Figure 5.

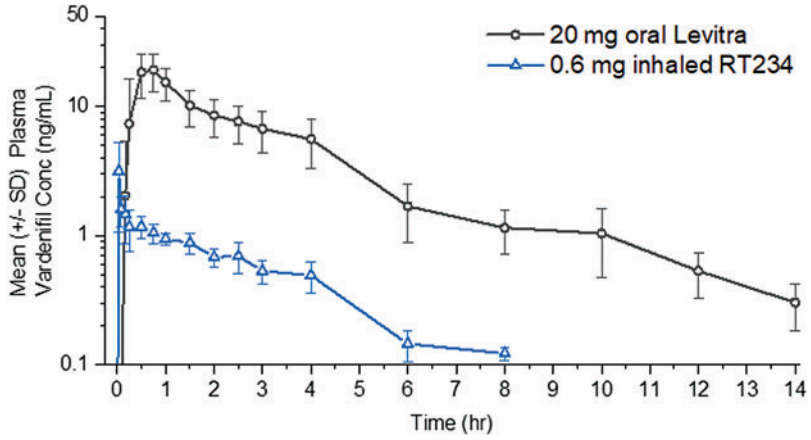

FIG. 4. Mean plasma vardenafil concentrations versus time after administration of single doses of 0.6 mg RT234 or 20 mg oral vardenafil (Levitra®) to healthy subjects Color images are available online.

TABLE 5. PLASMA VARDENAFIL PHARMACOKINETIC PARAMETERS AFTER ADMINISTRATION OF SINGLE DOSES OF VARDENAFIL AS 0.6 MG INHALED RT234 AND 20 MG ORAL LEVITRA TABLETS TO HEALTHY SUBJECTS

	Mean (CV%) plasma vardenafil pharmacokinetic parameter values			
Treatment	t_{max}[a] *(h)*	C_{max} *(ng/mL)*	$AUC_{(0-last)}$ *(h · ng/mL)*	$AUC_{(0-inf)}$ *(h · ng/mL)*
0.6 mg RT234 (*N* = 5)	0.03	3.178 (65)	4.052 (18)	4.348 (18)
20 mg oral Levitra (*N* = 5)	0.75	21.16 (30)	53.15 (29)	54.36 (29)
20 mg oral Levitra/0.6 mg RT234	25	6.7	13.1	12.5
	Mean (CV%) plasma vardenafil bioavailability parameter values after administration of 2.4 mg RT234			
		C_{max} *(ng/mL)*	$AUC_{(0-last)}$ *(h · ng/mL)*	$AUC_{(0-inf)}$ *(h · ng/mL)*
2.4 mg RT234 (*N* = 6)		8.467 (40)	18.2 (15)	18.67 (14)
20 mg oral Levitra/2.4 mg RT234		2.5	2.9	2.9

[a]Median t_{max} displayed.

Discussion

The starting dose of Levitra for the treatment of erectile dysfunction (ED) is 5 mg. The dose is decreased (2.5 mg) or increased (10 or 20 mg) based on the patient's response (safety and efficacy) to the initial dose.[11] In a randomized, double-blind, placebo-controlled study, Jing et al.[12] demonstrated that twice daily administration of a 5 mg tablet of Levitra to Chinese subjects with PAH led to significant improvements in pulmonary hemodynamics and exercise capacity as demonstrated by a 69 m improvement in 6 minute walking distance at 24 weeks. These changes were accompanied by statistically significant improvements in New York Heart Association (NYHA) functional class.

The current study in healthy subjects evaluated the safety, tolerability, and PKs of single and multiple doses of a dry powder formulation of vardenafil (RT234) administered through oral inhalation. After administration of a single ascending dose of RT234, absorption was very rapid and AUC increased linearly to the maximum dose of 2.4 mg. Between-subject variability in AUC were much less than is observed for the oral tablet.[10,11] This is likely due to reductions in first-pass effects following inhalation.

In contrast to current approved oral PDE5i formulations of sildenafil and tadalafil, where systemic absorption occurs over 1–2 hours,[13] systemic absorption of RT234 peaked in plasma within 2 minutes. The rapid absorption of vardenafil through the lungs should also lead to a rapid hemodynamic response. Indeed, measures of pulmonary hemodynamics following right heart catheterization in subjects with PAH demonstrated a comparable pulmonary hemodynamic effect for the 0.6 mg RT234 dose relative to a 20 mg oral Levitra tablet, although with greater pulmonary selectivity and improved oxygenation (Study ACTRN12618001077257).[14,15] A 10%–20% improvement in pulmonary vascular resistance was observed within 5 minutes following pulmonary administration of RT234, with a peak effect within 15–30 minutes.[14] The rapid PK and pharmacodynamic

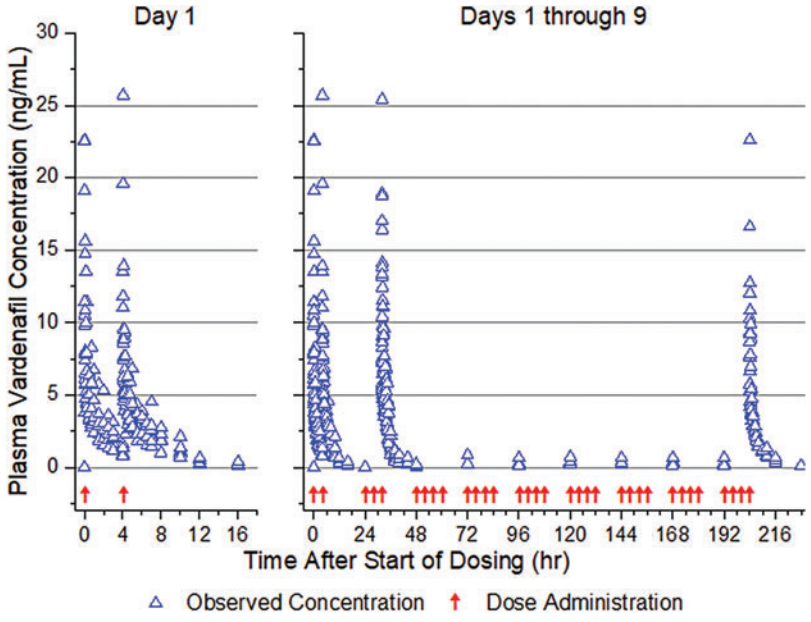

FIG. 5. Scatter plot of plasma vardenafil concentrations versus time from all subjects receiving repeated doses. Color images are available online.

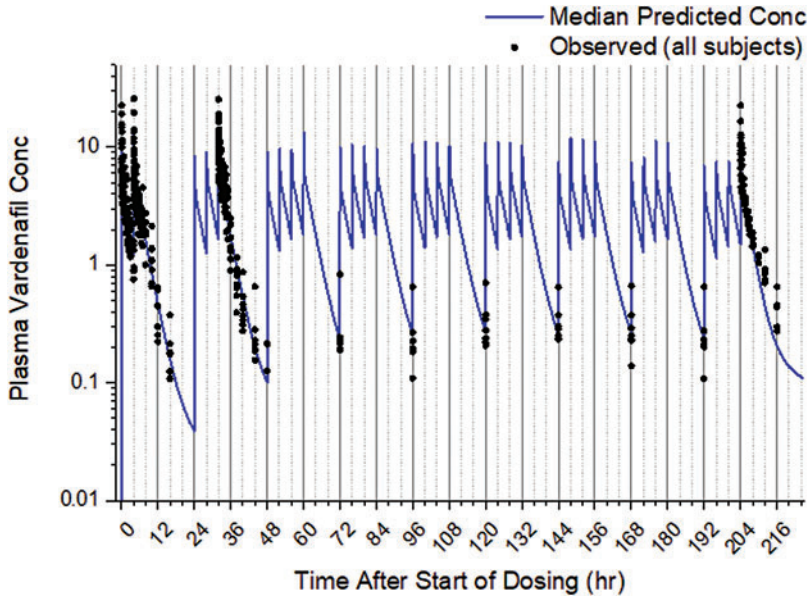

FIG. 6. Observed and model-predicted plasma vardenafil concentrations versus time from all subjects receiving repeated doses. Color images are available online.

responses observed with RT234 are critical features for an as-needed vasodilator.

Compared with other PDE5i, vardenafil has an extended half-life on the catalytic receptor of the PDE5 enzyme (i.e., 1–2 hours vs. 3 and 7 minutes, for sildenafil and tadalafil, respectively).[16,17] The extended receptor half-life may provide a prolonged hemodynamic effect, enabling PAH patients to complete activities away from home. Indeed, improvements in mean pulmonary artery pressure were maintained for at least 3 hours in a thromboxane-based pulmonary hypertension model in dogs, following pulmonary administration of vardenafil.[18]

The extended time on the receptor allows for prolonged efficacy to be achieved without the need to maintain systemic drug concentrations of the PDE5i. Indeed, efficacy in ED patients is maintained for oral vardenafil even when systemic drug levels are below therapeutic levels.[19,20] In addition to the extended receptor half-life, the low oral bioavailability (~15%), high protein binding (>95%), and rapid systemic clearance make vardenafil an ideal vasodilator for targeting the pulmonary vasculature and minimizing systemic and gastrointestinal exposure.[21] The improved lung targeting of vardenafil leads to large decreases (33-fold) in nominal dose to achieve a comparable pharmacodynamic effect.[14] This leads to decreases in systemic exposure by about one order of magnitude, and gastrointestinal exposure by about two orders of magnitude relative to oral Levitra.

RT234 was generally well tolerated at single doses up to 2.4 mg, and multiple doses every 4 hours up to 9.6 mg/day, with no serious or severe TEAEs. The most common TEAE was headache. No TEAEs indicative of pulmonary irritation or inflammation (e.g., bronchospasm, wheezing, dyspnea, throat irritation) were observed. No clinically significant alterations in vital signs, ECGs, pulmonary function testing, or laboratory-related parameters were identified with administration of vardenafil inhalation powder throughout the dose range from 0.2 to 9.6 mg/day. The current findings suggest that at the anticipated therapeutic dose (~0.6 mg), RT234 has a safety and PK profile consistent with that of an as-needed vasodilator.

TABLE 6. PLASMA VARDENAFIL PHARMACOKINETIC PARAMETERS BY DAY AND DOSE NUMBER

Day and dose number	*Mean (95% CI[a]) plasma vardenafil pharmacokinetic parameters*				
	t_{max}[b] *(h)*	C_{max} *(ng/mL)*	$AUC_{(0-4)}$ *(h · ng/mL)*	$AUC_{(0-last)}$ *(h · ng/mL)*	$AUC_{(0-inf)}$ *(h · ng/mL)*
Day 1, dose 1	0.090	21.44 (16.76–29.59)	12.62 (10.85–15.62)	ND	ND
Day 1, dose 2	0.075	18.92 (15.24–23.44)	15.32 (13.37–18.33)	21.32 (18.87–24.97)	21.85 (19.29–25.63)
Day 2, dose 3	0.080	15.58 (12.40–18.85)	16.02 (13.97–18.68)	22.58 (20.54–25.51)	23.54 (20.87–27.51)
Day 9, dose 4	0.080	14.52 (11.64–21.18)	15.43 (13.40–19.76)	23.69 (20.24–31.64)	25.29 (21.95–32.67)

[a]Bias-corrected Bayesian bootstrap CI.
[b]Median t_{max} from observed values.
ND, not determined; CI, confidence interval.

Conclusions

The PK results of this study indicate significant, predictable, and dose-proportional vardenafil systemic exposure that supports the assessment of safety and tolerability of RT234 administered using dose levels and dosing regimens suitable for further clinical development of an as-needed vasodilator. Comparative plasma vardenafil bioavailability data provide scientific justification for reliance on FDA findings of safety for Levitra tablets as per the 505(b)(2) development pathway.

Author Disclosure Statement

The authors declare they have no competing financial interests.

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Supplementary Material

Supplementary Data

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