

Online data supplement

Characterization of cough evoked by inhaled treprostinil and treprostinil palmitil.

Richard W. Chapman ¹, Michel R. Corboz ¹, Carlos Fernandez ¹, Eugene Sullivan ¹, Andy Stautberg ¹, Adam J. Plaunt ¹, Donna M. Konicek ¹, Vladimir Malinin ¹, Zhili Li ¹, David Cipolla ¹ and Walter Perkins ¹.

¹. Inmed Incorporated, Bridgewater, NJ, USA

PRE-CLINICAL STUDIES IN GUINEA PIGS AND RATS

1. Methods

a. Animals

Experiments were performed in male Hartley guinea pigs (300-460 g) and male Sprague Dawley rats (300-400 g) and were housed in temperature (21°C) and humidity-controlled conditions. The animals were acclimated to the laboratory surroundings for at least 3 days before study with up to 7 days acclimation for studies involving plethysmography. All experiments were performed in accordance with the Canadian Council for Animal Care (CCAC) and followed the principles of Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58) and current OECD/MHLW and ICH guidelines.

b. Inhalation exposures, cough, ventilation and Penh measurements in guinea pigs

The guinea pigs were placed in a whole-body plethysmograph for the measurement of ventilation (tidal volume, respiratory rate and minute volume), Penh and cough using established techniques [1, 2]. Cough was measured from plethysmograph recordings showing a large inspiration followed by a large expiration and confirmed by manual observations, video recordings and cough sounds [1, 2]. The ventilation, Penh and cough data were measured during a 15-min baseline period before the exposure to the aerosolized drug and represented as an average value over this time period. Measurements were then obtained over 5-minute intervals during the exposure to aerosolized test article and then every 15 minutes after test article administration for up to 2 hours post dose.

Treprostinil (TRE) or phosphate buffered saline (PBS) were given by nebulization using an Aeroneb Pro vibrating mesh nebulizer (Aerogen, CA, USA). The duration of nebulization was 10 minutes. Air was circulated through the plethysmograph using a compressed gas source (21% O₂/balance N₂) that entered at a flow rate of 2 L/min through a port in the top of the plethysmograph and exited from two ports at the bottom; a primary outlet port and a filter sampling port

that had flows of 1.6 and 0.5 L/min, respectively. The nebulized drugs were introduced to the air inflow with a T-connector [1].

For the aerosolization of treprostinil palmitil inhalation powder (TPIP) and the mannitol vehicle control formulation, a Vilnius Aerosol generator (VAG) was connected to the top of the plethysmograph and air from a compressed gas source was introduced into the VAG at a flow rate of 4.5 L/min. The dispersed dry powder aerosol was combined with 1 L/min of humidified air (30% humidification) (total air inflow of 5.5 L/min) to facilitate aerosol delivery to the plethysmograph and minimize the potential for static adhesion of particles. The typical humidity of the supplied air was measured around 30%. The generator output from the VAG was 1, 0.75 and 0.5 Volts for TPIP and 1 Volt for mannitol vehicle with each VAG output given for 15-min to deliver 3 different doses of the drugs, with a higher dose being delivered at the higher voltage. A vacuum flow of 8 L/min was established at the bottom of the plethysmograph such that the air and aerosols entered the top and exited the bottom of the system. A separate vacuum source of 0.5 L/min was connected to the filter that was attached to a port in the plethysmograph to sample the treprostinil palmitil (TP) concentration.

c. Prostanoid, tachykinin and bradykinin receptor antagonists and meclofenamic acid in guinea pigs

The following compounds were evaluated against TRE induced cough: prostanoid IP (RO1138452), EP₁ (ONO-8711), EP₂ (PF-04418948), EP₃ (L-798,106), DP₁ (BWA868C), tachykinin NK₁ (CP99994), and bradykinin B₂ (HOE 140) receptor antagonists and the cyclooxygenase inhibitor, meclofenamic acid. The compounds were administered intraperitoneally, 30-min before TRE administration (Table 1) except for HOE 140 that was given by aerosol (1 mg/mL) for 10-min with a 10 min pretreatment time before TRE administration. The doses, concentrations and pretreatment times and their vehicle controls were selected based on data reported in previous studies [3-6].

Table 1. Doses, concentration solutions and volume of injection with intraperitoneal compounds.

Drug Class	Antagonist (A) or inhibitor (I)	Administrated Dose (mg/kg)	Solution Concentration (mg/mL)	Volume (mL/kg)
Prostanoid	IP (A)	10	5	2
Prostanoid	EP ₁ (A)	10	5	2
Prostanoid	EP ₂ (A)	5	1	5
Prostanoid	EP ₃ (A)	10	2	5
Prostanoid	DP ₁ (A)	10	1	10
Tachykinin	NK ₁ (A)	10	1	10
Cyclooxygenase	Meclofenamic acid (I)	1	1	1

All compounds were given intraperitoneally 30 min before TRE administration.

I. RO1138452 (IP antagonist)

The IP antagonist was resuspended in 100% DMSO by vortexing to a concentration of 10 mg/mL. A 2-fold dilution was made into 0.9% saline to obtain a final concentration of 50% DMSO (v/v) and 5 mg/mL of the IP antagonist.

II. ONO-8711 (EP₁ antagonist)

The EP₁ antagonist was supplied in methyl acetate which was evaporated to a thin film under flowing nitrogen. The thin film of EP₁ antagonist was dissolved in 100% DMSO by vortexing and sonicating to a concentration of 50 mg/mL. A 10-fold dilution was made in isotonic saline containing 50 mg/mL hydroxypropyl- β -cyclodextrin (HPBCD) and 40% DMSO (v/v) and vortexed to generate a final concentration of 5 mg/mL of EP₁ antagonist in 50 mg/mL HPBCD, 50% DMSO (v/v), and 0.9% saline (w/v).

III. PF-04418948 (EP₂ antagonist)

The EP₂ antagonist was dissolved in 100% DMSO, by vortexing, yielding a concentration of 25 mg/mL. A 25-fold dilution was made in isotonic saline containing methylcellulose (400 cP; 0.5% w/v), Tween 80 (0.2% v/v) and DMSO (16% v/v) in isotonic saline and vortexed to obtain a final concentration of 1 mg/mL of the EP₂ antagonist in 20% DMSO (v/v), 0.5% methylcellulose (w/v), 0.2% Tween 80 (v/v) and 0.9% saline (w/v).

IV. L-798,106 (EP₃ antagonist)

The EP₃ antagonist was dissolved in 100% DMSO, by vortexing, yielding a concentration of 10 mg/mL. A 5-fold dilution was made in isotonic saline containing methylcellulose (400 cP; 0.5% w/v) and Tween 80 (0.2% v/v) to obtain a final concentration of 2 mg/mL of the EP₃ antagonist in 20% DMSO (v/v), 0.5% methylcellulose (w/v), 0.2% Tween 80 (v/v) and 0.9% saline (w/v).

V. BW A868 (DP₁ antagonist)

The DP₁ antagonist is supplied in 100% ethanol at 10 mg/mL. A 10-fold dilution was made into isotonic saline to obtain a final concentration of 1 mg/mL of the DP₁ antagonist in 10% ethanol (v/v).

VI. CP 99994 (NK₁ antagonist)

The NK₁ antagonist was dissolved in isotonic saline (0.9% w/v) at a final concentration of 1 mg/mL.

VII. HOE 140 (B₂ antagonist)

The bradykinin B₂ antagonist was dissolved in PBS at a final concentration of 1 mg/mL and given by aerosol for 10 min.

VIII. Meclofenamic acid (Cyclooxygenase inhibitor)

Meclofenamic acid was dissolved in isotonic saline (0.9% w/v) at a final concentration of 1 mg/mL.

d. Laryngeal and cardiovascular reflexes in rats

Male Sprague Dawley rats (300-400 g) were anesthetized with an intraperitoneal injection of urethane (1.3 g/kg) and the trachea was exposed to isolate the larynx. The trachea was then sectioned into an upper and a lower airway segment. A catheter was inserted into the lower airway and positioned just above the carina and a second catheter was inserted into the upper airway segment and positioned just in front of the larynx. An oral tube was introduced through the mouth to facilitate the free passage of aerosols through the laryngeal airway and out through the oral tube. A catheter was placed in the femoral artery for the measurement of mean arterial blood pressure (MAP) and heart rate (HR) and a pneumotachograph was connected to the tracheal tube in the lower airway segment through which the animal breathed. Respiratory measurements were obtained of the respiratory rate, tidal volume and the duration of expiration (T_E) from which the apneic ratio (T_E after nebulization/ T_E before nebulization) was measured [7-9]. A pulse oximeter was placed on the front paw to measure the peripheral capillary oxygen saturation (SpO_2). The number of swallows were enumerated by the presence of laryngeal elevation [8]. Compounds were delivered directly through the laryngeal catheter by nebulization for 20 seconds using an Aeroneb Pro vibrating mesh nebulizer. Test articles evaluated included isotonic (0.9%) saline, hypertonic (1.5, 3.5 and 7%) saline, citric acid (0.01-1 M) or TRE (0.1 – 15 mM).

For intravenous (i.v.) administration, a catheter was inserted into the femoral vein and i.v. injections (1.3 mL/kg volume) of PBS, TRE (10-3000 μ g/kg) and capsaicin (1.25 μ g/kg) were sequentially administered into the same rat. Capsaicin at an i.v. dose of 1.25 μ g/kg was used as a positive control for induction of apnea and a fall in systemic arterial blood pressure (SAP) in rats [10].

e. Laryngeal reflexes in guinea pigs

Guinea pigs were anesthetized with an intraperitoneal injection of urethane (1.3 g/kg) and the trachea was exposed to isolate the larynx. Catheters were placed into the upper segment of the trachea for the delivery of drugs to the larynx and into the lower segment of the trachea through which the animal breathed. Details of this procedure are described above for experiments in rats. A pneumotachograph was connected to the lower tracheal segment from which respiratory measurements were obtained of the respiratory rate, tidal volume and the duration of expiration (T_E) from which the apneic ratio (T_E after nebulization/ T_E before nebulization) was measured [9]. The number of swallows were enumerated by the presence of laryngeal elevation [8]. Compounds were delivered directly through the upper tracheal catheter by nebulization using an Aeronex Pro vibrating mesh nebulizer. Reflexes measured from the laryngeal challenge with nebulized isotonic saline, citric acid (1 M) or TRE (0.1 mM) included cough, apneic ratio (T_E after nebulization/ T_E before nebulization) and swallow. Saline and citric acid were given for 20 seconds and TRE was given for 3 minutes.

f. Bronchospasm/bronchodilation in guinea pigs

Guinea pigs were anesthetized with a mixture of 1.5 to 2% isoflurane in oxygen, a tracheal catheter inserted and connected to a positive pressure rodent respirator at settings of 50 breaths/min and tidal volume of 3 mL. Pulmonary insufflation pressure (PIP) was continuously recorded as described previously [11]. A catheter was inserted into the jugular vein for the IV injection of histamine di-hydrochloride (1.5 μ g/kg) to induce a bronchospasm. Test articles of TRE (1.5 and 3 μ g/kg, pulmonary dose) or PBS were delivered directly through the tracheal catheter via an Aeronex vibrating mesh nebulizer interposed in the inspiratory limb of the ventilator. PIP was measured immediately before and during the peak increase (within 1 minute) after IV histamine with histamine challenges performed at times of 15 min before and 5, 60, 120 and 180 min after the PBS or TRE administration. The percentage increase in PIP was measured

for each histamine challenge as previously described [11]. Statistically significant ($P < .05$) differences were determined by repeat paired or un-paired t-tests.

2. Results

a. Ventilation and Penh with TPIP and vehicle in guinea pigs

The results in Tables 2 and 3 demonstrate no significant effects of TPIP (35.8 µg/kg) or dry powder mannitol vehicle on respiratory frequency, tidal volume, minute volume or Penh in guinea pigs.

Table 2: Average respiratory parameters from guinea pigs exposed to dry powder mannitol vehicle.

Time (min)	f avg	±SEM	TV avg	±SEM	MV avg	±SEM	Penh avg	±SEM
0	196	13.5	2.48	0.415	481	106.5	1.168	0.242
5	193	9.5	2.23	0.295	426	70	1.087	0.198
10	191	7	2.12	0.195	387	41.5	1.008	0.142
15	177	8.5	2.07	0.14	344	27.7	0.936	0.151
30	187	15.5	2.01	0.13	350	49	1.042	0.132
45	173	15	2.10	0.245	322	41	1.264	0.391
60	163	3	2.05	0.235	260	27	1.808	0.270
75	166	7	2.20	0.155	332	43.5	1.144	0.164
90	149	5.5	1.95	0.185	244	29.5	1.854	0.283
105	175	3.5	1.89	0.16	284	40.5	1.453	0.199
120	164	21.5	2.21	0.13	317	67	1.756	0.321
135	167	10	1.93	0.10	263	32	1.649	0.435

Abbreviations: f avg, respiratory rate average; TV avg, tidal volume average; MV avg, minute volume average; Penh avg, enhanced pause average; SEM, standard error of the mean.

Time = 0 min (baseline before test article).

Time = 5, 10 and 15 min (during the 15 min of test article exposure).

Time = 30-135 min (after test article exposure).

Table 3: Averaged respiratory parameters from guinea pigs exposed to TPIP (35.8 µg/kg).

Time (min)	f avg	±SEM	TV avg	±SEM	MVavg	±SEM	Penh avg	±SEM
0	194	15	2.09	0.13	375	34.5	1.057	0.149
5	185	16.5	2.10	0.125	358	49.5	1.208	0.215
10	174	11.5	2.17	0.200	346	40.5	1.253	0.310
15	194	24.5	2.16	0.44	346	67.5	1.380	0.473
30	173	16	2.40	0.495	392	104	1.517	0.205
45	191	30.5	1.87	0.405	307	48.5	1.984	0.169
60	185	22.5	1.85	0.315	305	36	1.921	0.304
75	186	23.5	1.93	0.43	307	55.5	2.332	0.416
90	188	29.5	2.02	0.39	329	55	2.194	0.458
105	178	20	2.05	0.36	323	61	1.973	0.227

120	172	24.5	2.50	0.86	275	29	2.458	0.269
135	169	19	1.93	0.225	293	25	2.037	0.256

Abbreviations: f avg, respiratory rate average; TV avg, tidal volume average; MV avg, minute volume average; Penh avg, enhanced pause average; SEM, standard error of the mean.

Time = 0 min (baseline before test article).

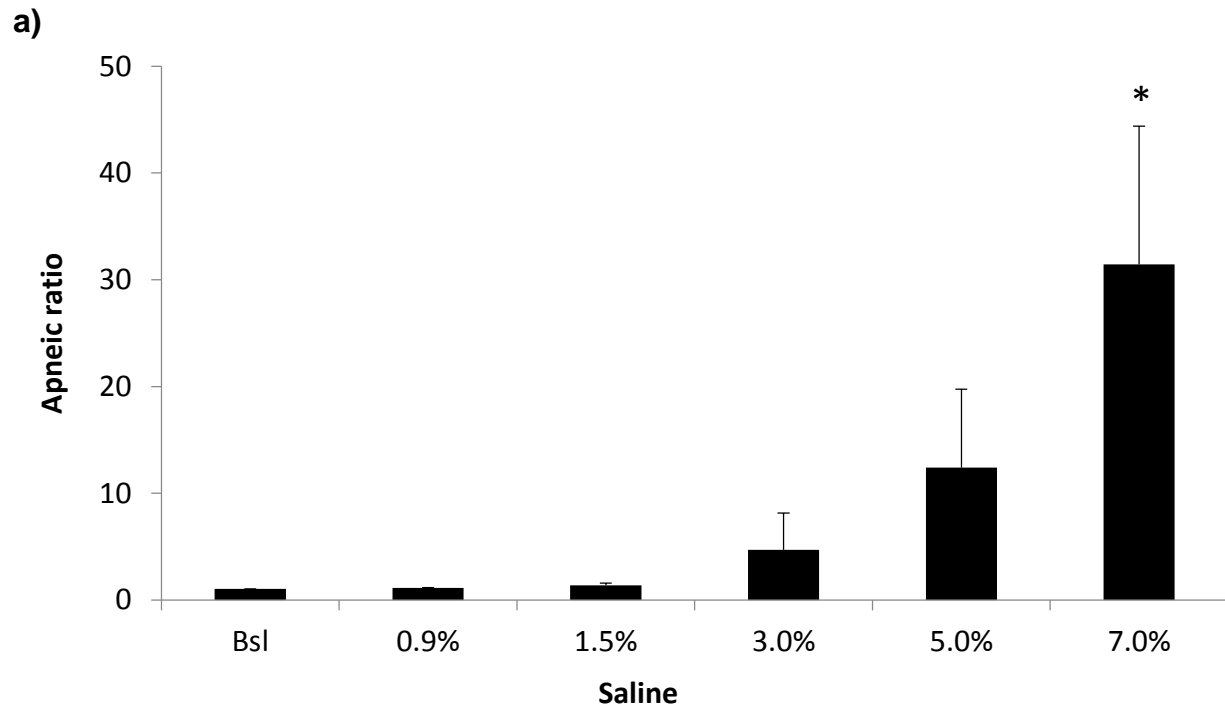
Time = 5, 10 and 15 min (during the 15 min of test article exposure).

Time = 30-135 min (after test article exposure).

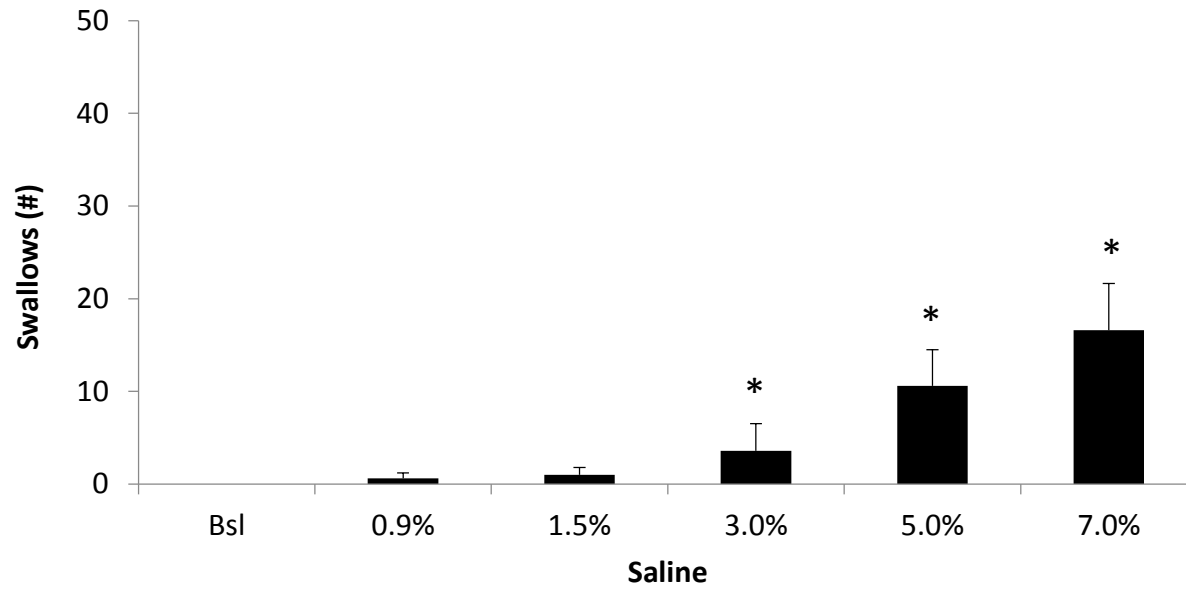
b. Laryngeal and cardiovascular reflexes in rats

Nebulized hypertonic saline administered to the isolated laryngeal airway of rats produced apnea and swallows that increased as a function of the saline concentration (Figure 1)

Figure 1: Laryngeal reflexes of apnea and swallow were induced by nebulized hypertonic saline administered directly into the laryngeal airway of rats.



b)



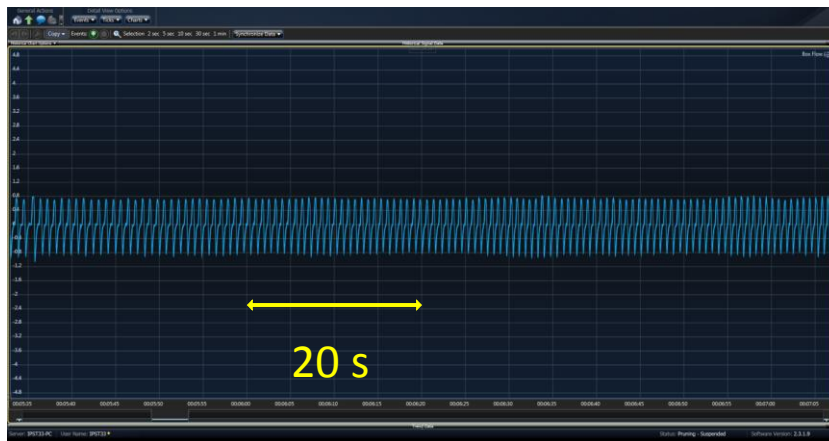
Values are the mean \pm SEM (n = 5) for a) the apneic ratio (T_E after nebulization of saline/ T_E before nebulization of saline) and b) the frequency of swallows. * $P < .05$ compared to baseline (Bsl).

c. Laryngeal and cardiovascular reflexes in guinea pigs

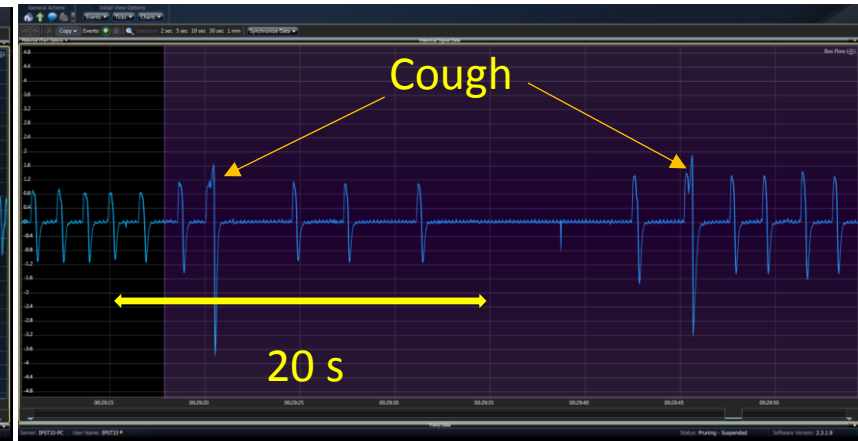
Nebulized citric acid (1 M for 20 s) administered to the isolated laryngeal airway of guinea pigs produced apnea, cough and increased the frequency of swallows whereas nebulized PBS administered for 20 s had no effects. Nebulized TRE (0.1 mM for 3 min) also had no effects to cause apnea, cough or swallowing (Figure 2).

Figure 2: Laryngeal reflexes of apnea, swallow and cough were induced by nebulized citric acid, but not TRE administered directly into the laryngeal airway of anesthetized guinea pigs.

a. Nebulized TRE



b. Nebulized Citric Acid, 1 M



	Apnea			Swallows
Treatment	Baseline T_E (s)	Treatment T_E (s)	Apneic Ratio	#
Saline	0.33	0.64	1.94	1

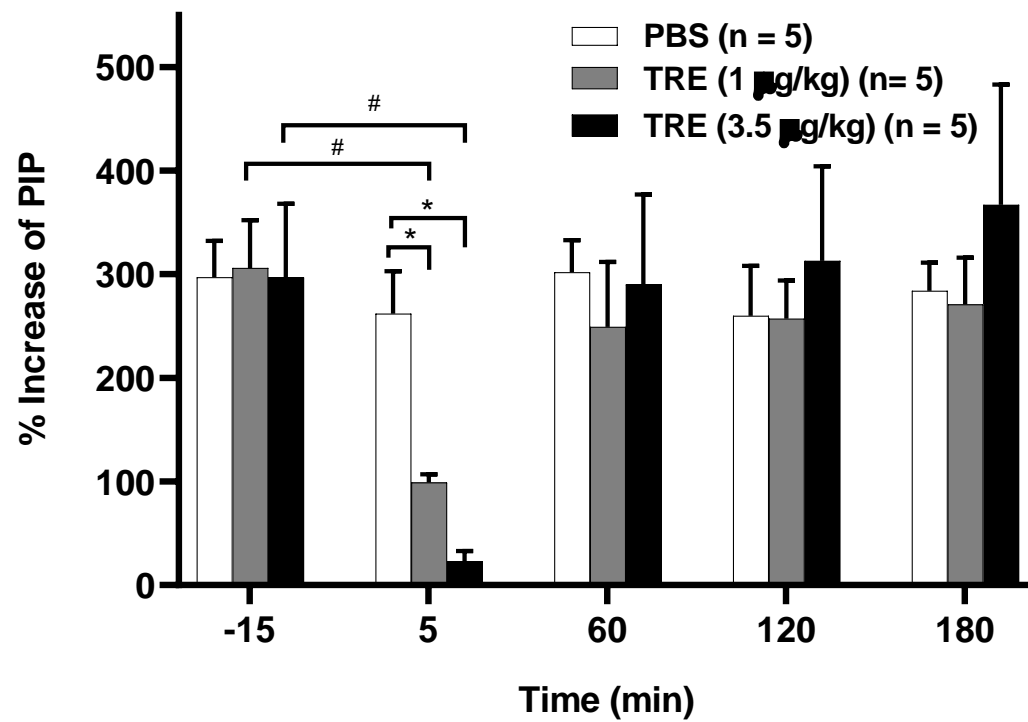
Citric Acid (1M for 20 s)	0.45	6.80	15.11	7
TRE (0.1mM for 3 min)	0.33	0.56	1.70	0

Representative chart recordings of the pulmonary airflow after a) nebulized saline and b) 1 M citric acid in a guinea pig to show the presence of apnea and cough with citric acid. The data in the table represent the mean values (n =6 per treatment group) baseline and treatment expiratory durations (T_E) in seconds, calculated apneic ratio (baseline T_E / treatment T_E), and frequency of swallows.

d. Bronchospasm/bronchodilation in guinea pigs

Intravenous histamine challenge (1.5 $\mu\text{g}/\text{kg}$) to guinea pigs increased PIP on average by approximately 300% from the baseline values (Figure 3). There was a significant ($P < .05$) and dose-dependent attenuation in the % increase in PIP due to histamine by 5 min but the effects had disappeared by 60 min (Figure 3). Inhaled PBS had no effects on the histamine bronchoconstriction. Furthermore, inhaled PBS or TRE (1 and 3.5 $\mu\text{g}/\text{kg}$) had no effects on the baseline PIP measured immediately before the 5 min histamine challenge and averaged 15.1 ± 0.84 , 15.3 ± 1.30 and 16.30 ± 0.58 cmH_2O , respectively (n = 5 per treatment). These results are consistent with published reports on the inhibition of airway smooth muscle contraction and bronchodilation with prostacyclin analogs [12, 13] with no evidence of bronchoconstriction following the administration of TRE directly to the airways and lungs.

Figure 3: Effect of nebulized treprostinil on histamine-induced bronchoconstriction in anesthetized guinea pigs.



Significantly different ($P < .05$) compared to values before treatment (- 15 min)

* Significantly different ($P < .05$) compared to PBS.

CLINICAL STUDY IN HEALTHY VOLUNTEERS

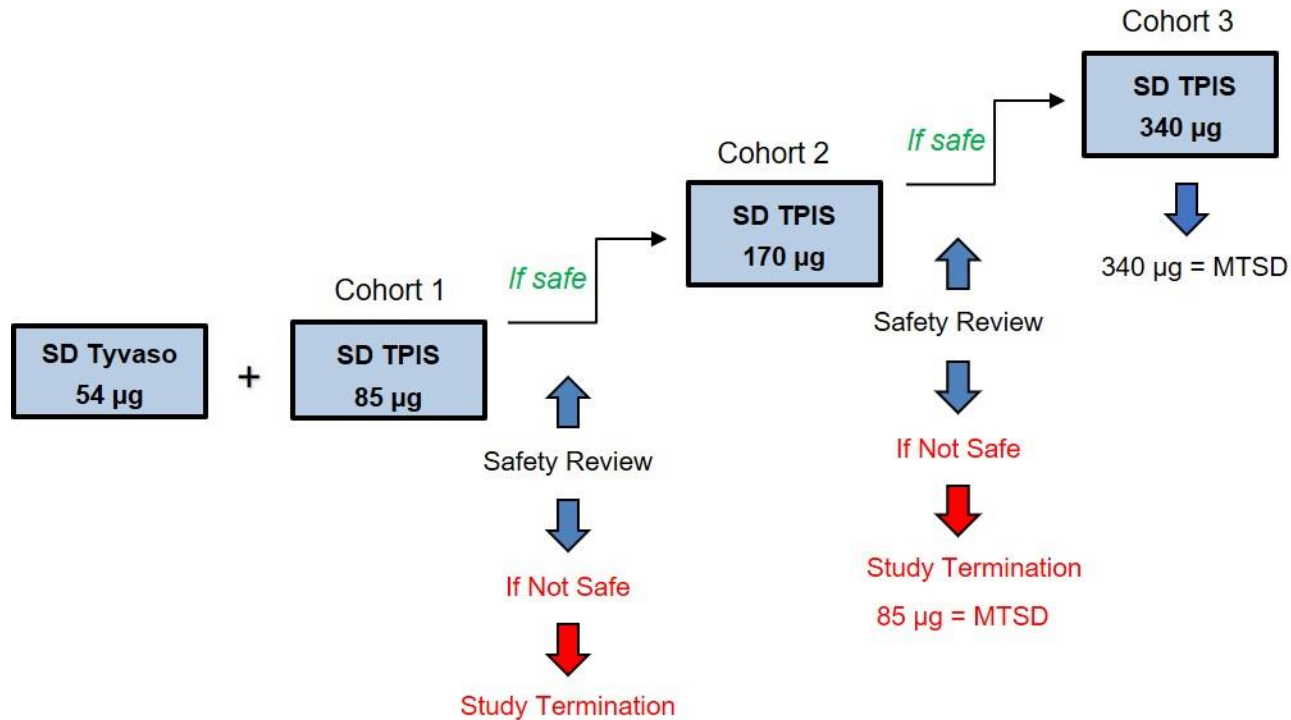
1. Methods

a. Subjects

Twenty-four subjects (19 males and 5 females) of mean age 28.7 years (range, 22 – 39), body weight of 78.18 kg (range, 56 – 98.3), and body mass index of 25.61 kg/m² (range, 21.3 – 31.4) were enrolled in the study. Nine subjects were Black or African American, eight subjects were White, four subjects were Asian and three subjects were classified as Other. The subjects were randomized into 3 cohorts with 8 subjects per cohort. All 24 subjects completed the study according to the protocol. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki under the Guidelines of the International Council for Harmonization (ICH) on Good Practice (GCP) (CPMP/ICH/135/95) [14, 15]. All patients gave signed written informed consent.

b. Study design

This was a randomized, double-blind, placebo controlled, single ascending dose study of TPIS with an open-label Tyvaso cohort performed in healthy human subjects to evaluate the pharmacokinetics (PK) and safety of TPIS. In the first cohort, all 8 subjects were exposed to a single dose of Tyvaso (54 µg TRE) and then, following a washout period of 24 hours, received, in a double-blind fashion, a single dose of TPIS (n = 6) at a dose level of 85 µg TP (molar equivalent to 54 µg TRE) or placebo (n = 2). Subjects in the next 2 cohorts were randomized in a double-blinded fashion to receive TPIS at dose levels of 170 µg or 340 µg or placebo in a 3:1 ratio. A schematic representation of the study design is illustrated below:



Abbreviations: TPIS-treprostinil palmitil inhalation suspension, SD-single dose, MTSD-maximum tolerated single dose.

c. Aerosol administration

Tyvaso® (United Therapeutics Inc, NC, USA) was administered according to the directions on the package insert [16]. TPIS and placebo were administered with a Phillips Micro nebulizer (Philips Innovation Service, MA, USA), a breath actuated, vibrating mesh nebulizer that requires approximately 6 inhalations to achieve a targeted dose.

d. Blood sampling and PK

Blood samples were collected through an indwelling catheter into a vacutainer with K₂EDTA before TPIS or placebo administration and then at times of 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hours post dose. The plasma concentrations of TRE and TP were measured by HPLC/MS/MS. The lower level of quantitation for TRE and TP were both 10 pg/mL.

e. Treatment-emergent adverse events (TEAEs)

The number and percentage of subjects with TEAEs are presented by treatment and dose group and are tabulated by body system and organ class. Placebo data from all cohorts were pooled.

2. Results

a. TEAEs with Tyvaso and TPIS

Overall, the most frequently reported TEAEs were cough, dyspnea and throat irritation, which were reported by 2 of 8 subjects (25%) that received Tyvaso, 9 of 18 subjects (50%) that received TPIS, and none of the subjects that received placebo. All instances of cough, dyspnea and throat irritation occurring in subjects that received TPIS were

at the higher dose levels. No subjects receiving 85 µg TPIS, the molar equivalent of 54 µg of Tyvaso, experienced these TEAEs.

Table 4. Treatment emergent adverse events (TEAEs) by system organ class and treatment

System Organ Class	Statistic	Tyvaso 54 µg (n = 8)	TPIS 85 µg (n = 6)	TPIS 170 µg (n = 6)	TPIS 340 µg (n = 6)	TPIS Overall (n = 18)	Placebo (n = 6)
Cardiac Disorders	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Nodal Arrhythmia	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0
Tachycardia	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Gastrointestinal Disorders	N (%)	0	1 (16.7)	0	2 (33.3)	3 (16.7)	0
Nausea	N (%) R	0	1 (16.7) R	0	2 (33.3) R	3 (16.7) R	0
Vomiting	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0
General Disorders And Administration Site Conditions	N (%)	1 (12.5)	0	0	0	0	0
Chest Discomfort	N (%) R	1 (12.5) R	0	0	0	0	0
Infections and Infestations	N (%)	0	1 (16.7)	0	0	1 (5.6)	0
Acute Sinusitis	N (%)	0	1 (16.7)	0	0	1 (5.6)	0
Injury, Poisoning And Procedural Complications	N (%)	0	0	0	0	0	1 (16.7)
Ligament Sprain	N (%)	0	0	0	0	0	1 (16.7) 1
Musculoskeletal And Connective Tissue Disorders	N (%)	0	1 (16.7)	0	1 (16.7)	2 (11.1)	0
Back Pain	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Muscle Spasms	N (%)	0	1 (16.7)	0	0	1 (5.6)	0
Musculoskeletal Stiffness	N (%)	0	1 (16.7)	0	0	1 (5.6)	0

Pain in Jaw	N (%)	0	1(16.7)	0	0	1 (5.6)	0
Nervous System Disorders	N (%)	1 (12.5)	0	1 (16.7)	2 (33.3)	3 (16.7)	0
Dysgeusia	N (%) R	0	0	1 (16.7) R	0	1 (5.6) R	0
Headache	N (%) R	1 (12.5)	0	0	2 (33.3) R	2 (11.1) R	0
Presyncope	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0
Syncope	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0
Respiratory, Thoracic And Mediastinal Disorders	N (%)	2 (25.0)	0	4 (66.7)	5 (83.3)	9 (50.0)	0
Cough	N (%) R	1 (12.5) R	0	3 (50.0) R	2 (33.3) R	5 (27.8) R	0
Dyspnea	N (%) R	1 (12.5) R	0	1 (16.7) R	3 (50.0) R	4 (22.2) R	0
Hemoptysis	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0
Throat Irritation	N (%) R	1 (12.5) R	0	2 (33.3) R	2 (33.3) R	4 (22.2) R	0
Surgical And Medical Procedures	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Venipuncture	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Vascular Disorders	N (%)	0	0	0	1 (16.7)	1 (5.6)	0
Orthostatic Hypotension	N (%) R	0	0	0	1 (16.7) R	1 (5.6) R	0

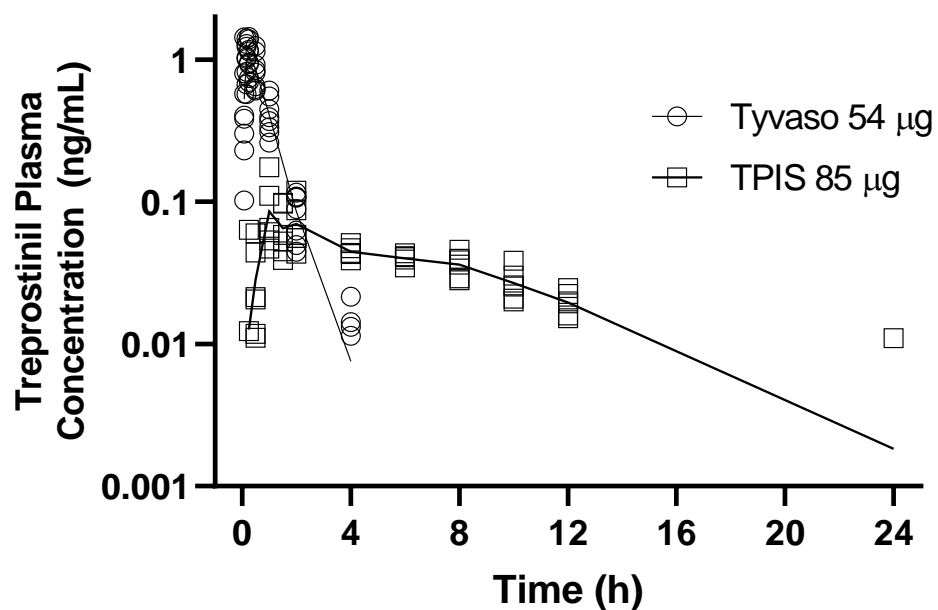
R = Related to treatment; n = Number of subjects dosed with each treatment (or any treatment as applicable); N = Number of subjects with characteristic; % = Calculated using the number of subjects treated with each treatment (or any treatment as applicable) as the denominator (100*n/N).

c. Pharmacokinetics of TPIS and Tyvaso

Comparison of the TRE PK parameters in Cohort 1 that received Tyvaso (54 µg) and TPIS (85 µg) in a crossover fashion reveals apparent differences in the absorption profiles of the 2 drugs (Figure 3, Table 5). PK exposure was somewhat higher after Tyvaso administration than after TPIS (AUC₀₋₂₄ was 872 pg.h/mL after Tyvaso and 614 pg.h/mL after TPIS). However, the plasma TRE C_{max} was 11-fold higher after Tyvaso (C_{max} = 958 pg/mL) compared to TPIS (C_{max} = 89 pg/mL), with the highest concentrations observed at 0.258 hours after Tyvaso compared with 1.02 hours after TPIS. This slower

rate of absorption resulted in a slower apparent half-life of TPIS as evidenced by the geometric $T_{1/2}$ of 5.69 hours after TPIS compared to 0.485 hours after Tyvaso.

Figure 3: Comparison of treprostinil in the plasma with individual subjects in cohort 1 receiving Tyvaso or TPIS.



Concentrations of treprostinil (TRE) in the plasma with Individual subjects receiving Tyvaso (54µg, n = 8) and TPIS (85µg, n = 6) in cohort 1. Values for TRE below the limit of quantification (10 pg/mL) were assigned a value of zero.

Table 5 Geometric mean (CV%) pharmacokinetic parameters stratified by cohort.

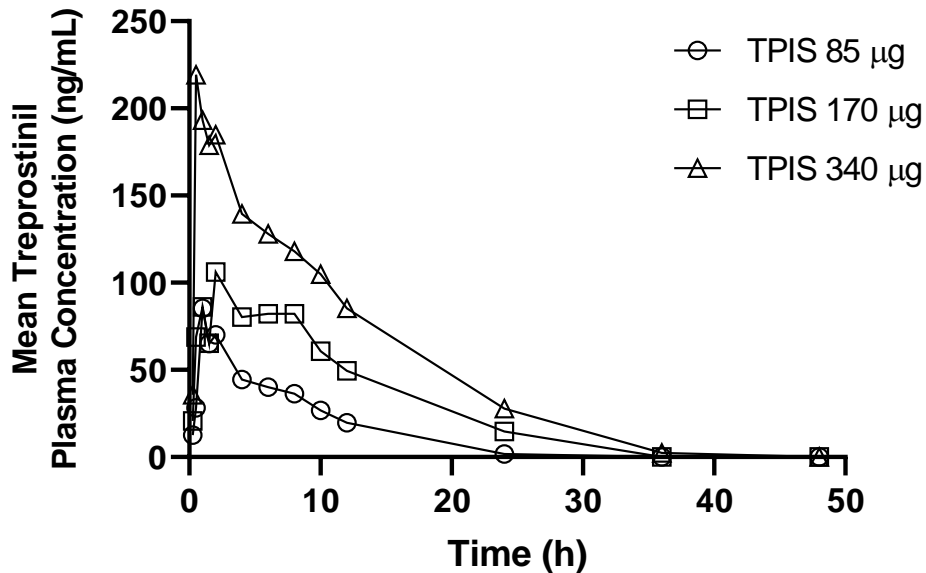
PK Characteristic	Cohort 1		Cohort 2 TPIS 170 µg N = 6	Cohort 3 TPIS 340 µg N = 6
	Tyvaso 54 µg (N = 8)	TPIS 85 µg (N = 6)		
C_{max} (pg/mL)				
Treprostinil	958 (26.5)	89.0 (49.2)	142 (24.9)	318 (33.1)
TP	—	0 (0 – 12.9) ^a	14.7 (0 – 23.0) ^a	34.2 (33.6)
T_{max} (h)^a				
Treprostinil	0.258 (0.183 – 0.300)	1.02 (1.00 – 2.03)	2.03 (1.02 – 8.00)	0.833 (0.517 – 2.02)
TP	—	2.01 (1.98 – 2.03)	2.02 (1.52 – 4.18)	2.09 (2.02 – 4.33)
AUC₀₋₆ (pg•h/mL)				
Treprostinil	872 (23.5)	297 (16.0)	468 (17.9)	897 (14.4)
TP	—	NC	76.3 (30.4)	148 (36.1)
AUC₀₋₂₄ (pg•h/mL)				
Treprostinil	872 (23.5)	614 (9.35)	1220 (21.4)	2160 (11.4)
TP	—	NC	222 (24.9)	318 (28.8)
AUC_{0-inf} (pg•h/mL)				
Treprostinil		674 (17.1)	1380 (24.3)	2490 (7.83)
TP	—	NC	194 (NC)	332 (30.3)
T_½ (h)				
Treprostinil	0.485 (23.7)	5.69 (59.7)	7.02 (22.7)	7.57 (31.7)
TP	—	NC	4.96 (NC)	4.57 (31.6)

AUC₀₋₆, area under the concentration-time curve from 0 to 6 hours; AUC₀₋₂₄, area under the concentration-time curve from 0 to 24 hours; AUC_{0-inf}, area under the concentration-time curve from 0 to infinity; TPIS, treprostinil palmitil inhalation suspension; C_{max}, maximum plasma concentration; N = number of subjects dose with each treatment; NC, not calculated; PK, pharmacokinetic; T_{max}, time to maximum plasma concentration; TP, treprostinil palmitil.

^a Median (Minimum – Maximum), all other values reported as geometric mean (CV%).

Treprostinil concentrations in plasma increased with increasing doses of TPIS (Figure 4). The plasma TRE C_{max} and AUC increased in a dose-dependent fashion with TPIS with no consistent change in plasma TRE T_{max} and a slight increase in the apparent plasma TRE $T_{1/2}$ (Table 5). The maximum plasma concentration of TP was barely above the level of detection after TPIS at 85 μg but increased as a function of TPIS dose at 170 and 340 μg (Table 5).

Figure 4: Mean Treprostinil Plasma Concentrations Following Single Dose TPIS Administration.



Mean concentrations of treprostinil (TRE) in the plasma with TPIS at doses of 85 μg ($n = 6$) in cohort 1, 170 μg ($n = 6$) in cohort 2 and 340 μg ($n = 6$) in cohort 3. Values for TRE below the limit of quantification (10 $\mu\text{g}/\text{mL}$) were assigned a value of zero.

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