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Early View

Original article

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Characterization of cough evoked by inhaled treprostinil and treprostinil palmitil.

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Take home message:

Cough induced by inhaled treprostinil and treprostinil palmitil involves the activation of prostacyclin (IP) receptors located on airway tachykinin nerves.

Abstract

Cough is induced by inhaled prostacyclin analogs including treprostinil (TRE), and, at higher doses, treprostinil palmitil (TP), a prodrug of TRE. In this report, we have investigated mechanisms involved with TRE- and TP-induced cough, using a dry powder formulation of TP (TPIP) to supplement previous data obtained with an aqueous suspension formulation of TP (TPIS).

Experiments in guinea pigs and rats investigated the prostanoid receptor subtype producing cough and whether it involved activation of sensory nerves in the airways and vasculature. Experiments involved treatment with prostanoid, tachykinin and bradykinin receptor antagonists, a cyclooxygenase inhibitor and TRE administration to the isolated larynx or intravenously.

In guinea pigs, cough with inhaled TRE (1.23 µg/kg) was not observed with an equivalent dose of TPIP and required higher inhaled doses (12.8 and 35.8 µg/kg) to induce cough. TRE cough was blocked with IP and tachykinin NK_1 receptor antagonists but not with EP_1, EP_2, EP_3, DP_1 or bradykinin B_2 antagonists or a cyclooxygenase inhibitor. TRE administered to the isolated larynx or intravenously in rats produced no apnea or swallowing, whereas citric acid, capsaicin and hypertonic saline had significant effects.

The mechanisms inducing cough with inhaled TRE likely involves the activation of prostanoid IP receptors on jugular C-fibers in the tracheobronchial airways. Cough induced by inhaled dry powder and nebulized formulations of TP occurs at higher inhaled doses than TRE, presumably due to the slow, sustained release of TRE from the prodrug resulting in lower concentrations of TRE at the airway sensory nerves.

Introduction

Treprostinil palmitil (TP) is a prodrug of the prostacyclin analog treprostinil (TRE) that is designed to provide prolonged pulmonary vasodilation via an inhaled route of administration [1]. It is in development for the treatment of pulmonary arterial hypertension (PAH) and has been studied as an inhaled aqueous suspension (TPIS) and as an inhaled dry powder (TPIP) formulation. In preclinical studies, a single dose of TPIS provided long-acting inhibition of pulmonary vasoconstriction with detectable concentrations of TP remaining in the lungs for up to 24 hours after administration [1, 2]. Consequently, the active drug TRE was also observed in plasma over the same extended time frame but at much lower levels compared to TPIS in the lung. In contrast, studies with inhaled TRE demonstrate its rapid elimination from the blood and lungs [1, 3, 4].

Cough and throat irritation are among the most common adverse events (AEs) of inhaled TRE administered to PAH patients [3-6]. The mechanisms underlying this side effect has not been previously elucidated. Conversely, TPIS does not cause cough at an equivalent inhaled dose to TRE that causes cough in both guinea pigs [1] and in human subjects [7, 8]. Here too, the factors involved in the lessened cough response to inhaled TP have not been defined.

In the present studies in guinea pigs and rats, we have investigated some of the potential mechanisms causing TRE-induced cough to identify the specific prostanoid receptor subtype(s) mediating TRE-induced cough, to elucidate the location and type of sensory nerve(s) producing this effect, to assess whether the presence of TRE in the blood was involved and to determine the actions of bradykinin or cyclooxygenase products that may be released by inhaled TRE. Furthermore, we evaluated whether inhaled TPIP acted like the other TP formulation (TPIS) and had a reduced propensity to cause cough compared to inhaled TRE, thereby distinguishing the contribution of the formulation from the prodrug itself. From these results, we have developed a hypothesis for why cough may be substantially lessened with administration of TP as either TPIS or TPIP. Some of these results have previously been reported in abstract form [7-11].

Materials and Methods

Details of the methods and supporting data from preclinical studies in guinea pigs and rats can be found in the online supplement. Also contained in the online supplement is a description of the methods and results from a Phase 1 pharmacokinetic (PK) and safety evaluation for TPIS in healthy human volunteers.

Compounds and materials

TPIP was produced at Bend Research Inc (Bend, OR, USA) as a formulated spray-dried powder and contained 1.5% of treprostinil palmitil (TP), 0.7% of DSPE-PEG2000, 68.2% of mannitol and 29.6% of L-leucine. A mannitol vehicle control was produced at Insmed (Insmed Incorporated, NJ, USA) and contained 0.7% of DSPE-PEG2000, 69.5% mannitol and 29.8% of L-Leucine. TRE and TP were obtained from Chirogate International (Taoyuan County, Taiwan, Republic of China). The following prostanoid, tachykinin, and bradykinin antagonists were purchased: RO1138452 (IP), ONO-8711 (EP_1), PF-04418948 (EP_2), L-798,106 (EP_3), BW A868C (DP_1) (Cayman Chemical, MI, USA), CP99994 (NK_1) (Tocris Bioscience, Bristol, UK), HOE 140 (bradykinin B_2) (MedChem Express, NJ, USA). Citric acid, capsaicin and meclofenamic acid were obtained from Sigma-Aldrich (St Louis, Mo, USA). Phosphate buffered saline (PBS) was acquired from Mediatech (Manassas, VA, USA). Details on preparation of solutions can be found in the online supplementary material.

Animals.

Male Hartley guinea pigs (300-460 g) and male Sprague Dawley rats (300-400 g) from Charles River Laboratories (St. Constant, Quebec, Canada) were housed in temperature (21°C) and humidity-controlled conditions with an acclimation period of 3 to 7 days. All experiments were performed in accordance with the Canadian Council for Animal Care (CCAC). Guinea pigs were acclimated to the plethysmograph once a day for a minimum of 3 consecutive days with a duration ranging from 30 min (for studies with TRE) min up to 2 hours (for TPIP).

Inhalation exposures in guinea pigs.

Guinea pigs were placed inside a whole-body plethysmograph (Data Sciences International, MN, USA) and exposed for 10 min to nebulized TRE or PBS using an Ultraneb Pro vibrating mesh nebulizer [1]. TPIP was administered for 15 min using a Vilnius Aerosol generator (VAG) (CH Technologies, NJ, USA) [11]. A glass filter was attached to a plethysmograph outlet port, connected to a vacuum flow of 0.5 L/min and the deposited drug (TRE or TP) collected over the duration of the aerosol exposure. The inhaled dose of TRE or TP was measured using an algorithm that has been previously described [12].

Cough in guinea pigs.

Cough was measured from plethysmograph recordings, confirmed by manual observations, video recordings and from cough sounds using a microphone [1, 13]. Tidal volume, respiratory rate, minute volume and enhanced pause (Penh) were also measured. Baseline data was obtained over 15 min before drug exposure, followed by measurements every 5 min during the aerosol exposure and every 15 min after drug administration for up to 2 hours post dose.

Prostanoid (IP, EP_1, EP_2, EP_3, and DP_1), tachykinin (NK_1), and bradykninin (B_2) antagonists and meclofenamic acid in guinea pigs.

Guinea pigs were injected intraperitoneally with prostacyclin (IP), prostaglandin E_2 (EP_1, EP_2 and EP_3), prostaglandin D_2 (DP_1) and tachykinin NK_1 receptor antagonists, meclofenamic acid or their respective vehicles 30 min before aerosolized TRE administration. The bradykinin B_2 antagonist was administered for 10 min by inhalation with a pretreatment time of 10 min before aerosolized TRE administration. The doses and concentrations of these antagonists and their vehicles were based upon data from previous studies [14-17].

Laryngeal and cardiovascular reflexes in rats and guinea pigs.

Rats and guinea pigs were anesthetized with urethane (1.3 g/kg) and drugs were administered to the isolated laryngeal airway as previously described [18,19]. Airflow through a lower tracheal catheter was measured with a pneumotachograph from which respiratory rate, tidal volume, minute ventilation, duration of expiration (T_E) and apneic ratio (T_E after nebulization/T_E before nebulization) were measured [18-20]. A catheter was placed into the femoral artery for the measurement of the systemic arterial blood pressures (SAP). The number of swallows were enumerated by the presence of laryngeal elevation [20].

Nebulized test articles (PBS, citric acid, and TRE) were delivered directly into the laryngeal catheter of rats for 20 seconds, and the apneic ratio, number of swallows, and changes in SAP were measured. In guinea pigs, laryngeal administration of PBS or citric acid was delivered for 20 seconds and TRE was administered for 3 minutes.

For intravenous (IV) administration (TRE, capsaicin, PBS) to rats, the apneic ratio and the mean arterial blood pressure (MAP) were obtained before and after injection of the test articles as previously described [21].

Data analysis and statistics

In guinea pig cough studies, statistically significant differences between TRE, TPIP and their respective vehicles was determined with a one-way analysis of variance (ANOVA) followed by Tukey's post-test analysis test using a GraphPadPrism 6 software package (GraphPad Software Inc., LaJolla, CA). For studies involving administration of prostanoid, tachykinin and bradykinin antagonists and meclofenamic acid, significant differences between drug treatments or their respective vehicles were determined using an unpaired t-test. In rats, drug treatment effects on the apneic ratio, frequency of swallows and SAP were compared and the vehicle controls using a t-test with repeated measures. Statistical significance was denoted as $P < .05$.

Results

TRE and TPIP on cough, ventilation and Penh in guinea pigs.

Inhaled TRE produced cough at doses of 3.30 and 1.23 µg/kg but not at a lower dose of 0.33 µg/kg (Figure 1 a). Much higher doses of TPIP (12.8 and 35.8 µg/kg) were required to elicit cough compared to TRE (Figure 1 b) and at a dose of 2.3 µg/kg no cough was observed. Cough was absent with the PBS and mannitol vehicles. The onset of cough with inhaled TRE occurred within the first 10 minutes of exposure whereas the onset of cough with TPIP was delayed, occurring between 17 to 35 minutes after the start of the exposure for individual guinea pigs (Figure 1 c). Cough with both TRE and TPIP was identical to that described with PGE_2 [22] and occurred in bouts of coughing with between 1 to 7 bouts per exposure. The cough response to TRE is illustrated in one guinea pig exposed to an inhaled TRE dose of 3.30 µg/kg where 7 bouts of coughing were observed (Figure 2).

TPIP had no effects on ventilation and Penh at each inhaled dose. There was a slight but not statistically significant increase in Penh over time with TPIP at 35 8 µg/kg, but the same trend was observed with the mannitol vehicle (Figure 3 and online supplement). The magnitude of this increase in Penh with TPIP and the mannitol vehicle is much less than that observed with inhaled bronchoconstrictor agents such as citric acid and capsaicin that reach Penh values as high as 1,000 along with labored breathing (unpublished observations).

Effect of prostanoid, tachykinin NK_1 and bradykinin antagonists and meclofenamic acid on TRE cough in guinea pigs.

TRE-induced cough was significantly (P <.05) inhibited by the IP receptor antagonist, RO 1138452 (10 mg/kg, i.p.) but not with the EP_1, EP_2, EP_3 and DP_1 antagonists (Figure 4 a). In contrast, RO 1138452 (10 mg/kg, i.p.) had no effect on citric acid-induced cough that induced 22 ± 17 coughs in the presence of RO 1138452 and 28 ± 17 coughs in the presence of the i.p. vehicle control.

TRE-induced cough was also significantly (P <.05) inhibited by the tachykinin NK_1 receptor antagonist, CP99994 (10 mg/kg, i,p.) whereas treatment with the bradykinin B_2 antagonist HOE 140 (1 mg/mL delivered as an aerosol) or the cyclooxygenase inhibitor, meclofenamic acid (1 mg/kg, i.p.) had no effects (Figure 4 b).

Laryngeal and cardiovascular reflexes with TRE in rats.

In anesthetized rats, nebulized citric acid (10-1000 mM) administered directly into the larynx increased the apneic ratio and increased the frequency of swallows (Figure 5 a, b). Similar results were found with increasing concentrations of nebulized hypertonic saline (online supplement). Nebulized TRE, at concentrations ranging from 0.1 to 1.5 mM, did not cause apnea or increase the frequency of swallows (Figure 5 a, b). These concentrations are 2 to 30 times above the concentrations that trigger cough in conscious guinea pigs (Figure 1).

Intravenous TRE (10-3000 ng/kg) had no effect on the apneic ratio and did not change the MAP (Figure 6 a, b) whereas a significant (P <.05) increase in the apneic ratio and decrease in the MAP was seen with i.v. capsaicin (1250 ng/kg). The doses of i.v. TRE used for this evaluation exceed those that induce pulmonary vasodilation in rats [23].

Discussion

In clinical studies, cough and throat irritation are the most frequently reported AEs with inhaled TRE and other inhaled prostanoid analogs such as iloprost [3-7]. To investigate the mechanisms involved with TRE cough, experiments were performed in guinea pigs, a species that responds to many of the tussive agents that cause cough in human subjects such as capsaicin, citric acid and prostaglandins [1, 13, 22, 24, 25]. Experiments were also performed in guinea pigs and rats to determine if TRE activates laryngeal or vascular reflexes that may be involved with cough following the local administration of TRE to the isolated larynx [18-20] or following intravenous administration [21]. The results from these studies demonstrate that TRE-induced cough involves the activation of IP receptors that are probably located on tachykinin-containing sensory nerves in the tracheobronchial airways. Furthermore, TPIP also induced cough in guinea pigs but required approximately a 7-fold (based upon the TRE equivalent inhaled dose) higher dose than inhaled TRE, consistent with observations for the TPIS formulation of TP.

Although TRE is classified as a prostacyclin analog, it binds to several prostanoid receptors with a rank order of potency of: EP_2 > DP_1 > IP > EP_1 > EP_3 > EP_4 and no binding (> 10 μM) to the $CRTH_2$ and FP receptors [1, 26]. To investigate the important prostanoid receptor subtype(s) involved with TRE-induced cough, guinea pigs were treated with potent and selective pharmacological antagonists for different receptor subtypes. TRE-induced cough was inhibited with RO 1138452, a selective IP antagonist [14] whereas the selective DP_1, EP_1, EP_2 or EP_3 antagonists [14-16] had no inhibitory effects. These results identify the IP receptor as the prostanoid receptor subtype mediating TRE cough. Importantly, there were no effects with EP_3 or DP_1 antagonists which are the prostanoid receptors involved with the cough response to PGE_2 and PGD_2 in guinea pigs [27, 28]. The selectivity of RO1138452 for TRE-induced cough was confirmed in our study as it had no effect against citric acid-induced cough.

Based upon previous studies that show prostacyclin analogs activate tachykinin-containing sensory nerves in various tissues, including airways [29-33] and from the data generated in the present study, it appears that cough induced by inhaled TRE likely involves the activation of IP receptors located on jugular C fibers in the tracheobronchial airways. The fact that TRE-induced cough was blocked by CP99994, a selective tachykinin NK_1 receptor antagonist [34] strongly suggests an involvement with the jugular C fibers as neurokinins are expressed in jugular C-fibers but not nodose C-fibers [35]. The location of the sensory nerves is probably in the tracheobronchial airways as topical administration of TRE to the isolated larynx of rats (Figure 5) and guinea pigs (on-line supplement) produced no reflex effects such as apnea, swallow and cough that occur with airway irritants such as citric acid or hypertonic saline [18-20]. Furthermore, intravenous TRE had no effects on the reflex apnea and hypotension that occurs with IV irritants in rats [21] which eliminates a vascular site of action. There were no changes in respiratory rate or Penh and no evidence of bronchoconstriction with inhaled TPIP or TRE in guinea pigs (Figure 3 and on-line supplement) which likely rules out the involvement of mechanosensitive airway nerves or nodose C-fibers [35, 36]. Furthermore, cough induced by inhaled TRE did not involve the secondary release of mediators such as bradykinin or products from cyclooxygenase metabolism as treatment with the bradykinin antagonist (HOE 140) or a cyclooxygenase inhibitor

(meclofenamic acid) had no effects. In summary, these results strongly suggest that TRE-induced cough involved the activation of IP receptors on jugular C fibers in the tracheobronchial airways, a conclusion that is consistent with previously published reports on the activation of non-adrenergic, non-cholinergic neurotransmission by non-prostanoid prostacyclin mimetics via activation of the IP_1-receptor subtype [33, 37].

As previously mentioned, there is excellent translation between humans and guinea pigs for cough induced by a variety of different respiratory irritants including capsaicin, citric acid and prostaglandin E_2 [1, 13, 22, 24, 25]. The results from this study indicate that inhaled TRE can be added to the list of tussive agents that show excellent translation between guinea pigs and humans for cough, as TRE produces cough in both guinea pigs [1, 9, 10] and humans [3, 4, 7], and experiments with the nebulized TP prodrug (TPIS) in both guinea pigs [1] and humans (on-line supplement) demonstrate that TP can cause cough, but only at significantly higher doses relative to TRE. Though inhaled mannitol, an excipient of TPIP, is a known tussive agent in humans [38], we ruled out a contribution of a mannitol effect on the response to TPIP as the mannitol vehicle produced no cough. The fact that both TPIS and TPIP produced cough at higher inhaled doses compared to TRE suggests it is unlikely that there were protective effects from these different formulations. Rather, the difference is most likely due to the relatively slow conversion rate of the prodrug to TRE resulting in the markedly lower peak concentrations of TRE for the same molar dose of TP. It remains to be seen if TPIP produces cough at a higher inhaled dose than inhaled TRE in humans and whether cough occurs in delayed bouts of coughing as was seen in guinea pigs.

There are several limitations to the conclusions reached in this study. First, the mechanistic studies were performed in guinea pigs so extrapolation of the results to humans is speculative. However, as discussed, there is excellent translation between guinea pigs and humans for the induction of cough to respiratory irritants, including TRE [1, 13, 22, 24, 25]. Furthermore, tachykinin receptor antagonists are now commercially available [39, 40], so it should be a straightforward task to determine if a tachykinin NK_1 receptor antagonist can block TRE cough in humans. Another potential limitation in this study pertains to studies in human subjects in which the overall plasma TRE exposure

(AUC) with TPIS (85 µg) was less than TRE (54 µg), even though the inhaled TRE doses were identical on a molar basis (after conversion of TP to TRE for TPIS). Therefore, the bioequivalence of TPIS and TRE could be in question. However, in guinea pigs and dogs, a lower incidence of cough occurs with TPIS compared to inhaled TRE [1] so we believe the lower level of respiratory AEs with TPIS in humans (on-line supplement) likely reflects the unique pharmacokinetics of the prodrug which delays presentation of TRE to the sensory nerves in the lungs and results in a significantly lower level of peak TRE exposure. Preclinical data with TPIP have found similar results of long-acting pulmonary vasodilation and a similarly low plasma TRE C_{max} with sustained concentrations of TP in the lungs over 12 to 24 hours (unpublished observations) so future studies of TPIP in humans should prove very enlightening should they confirm the findings of the preclinical data with respect to the cough response to equipotent doses of TP and TRE.

In conclusion, the results from this study suggest that activation of prostanoid IP receptors present on tachykinin-containing jugular C-fibers in the tracheobronchial airways mediate TRE-induced cough. As it appears that there is excellent translation between guinea pigs and humans in the cough response to inhaled TRE, it is likely that TPIP will behave in the same manner as TPIS and be associated with less cough in humans than inhaled TRE.

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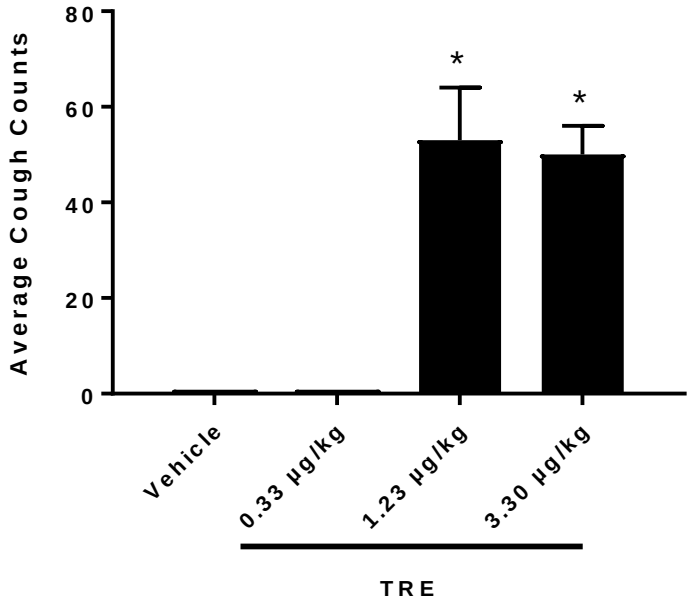
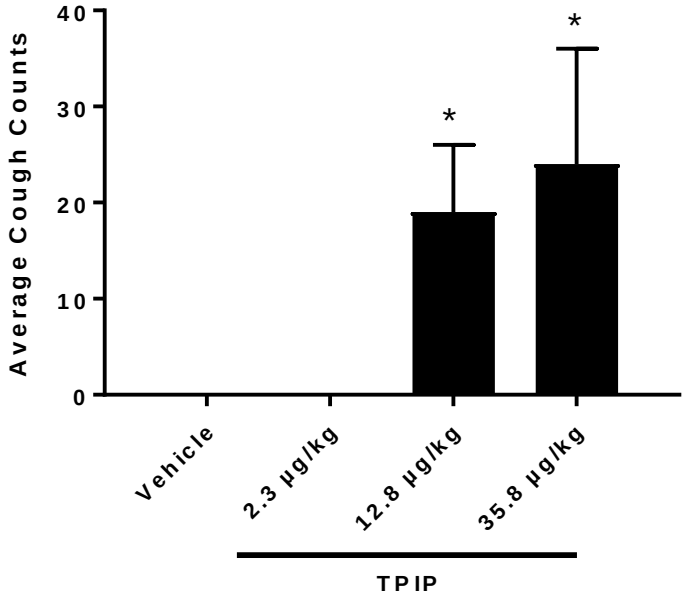
<u>FIGURES :</u> Characterization of cough evoked by inhaled treprostinil and treprostinil palmitil.

a)



b)



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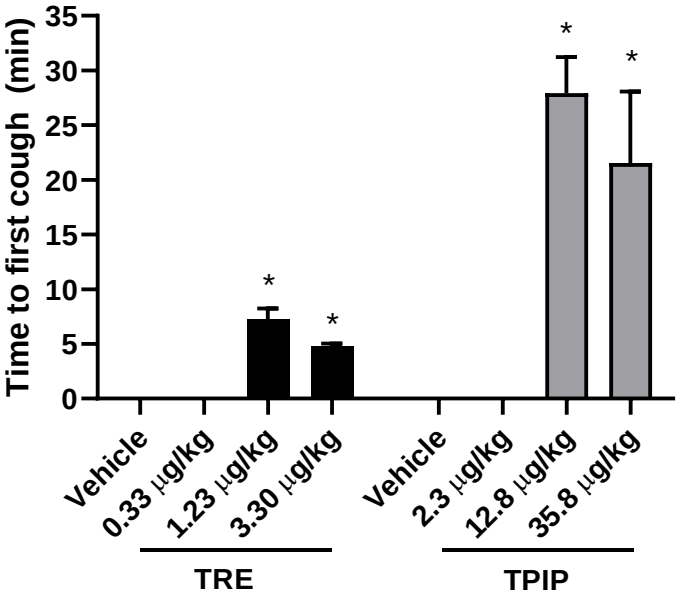


Figure 1. Cough induced by increasing doses of TRE and TPIP in guinea pigs. Values are the mean ± SEM cough counts following exposure to a) TRE (n = 4-10), b) TPIP (n = 4-6) and c) the time to the first cough for both TRE and TPIP. * $P < .05$ compared to vehicle.

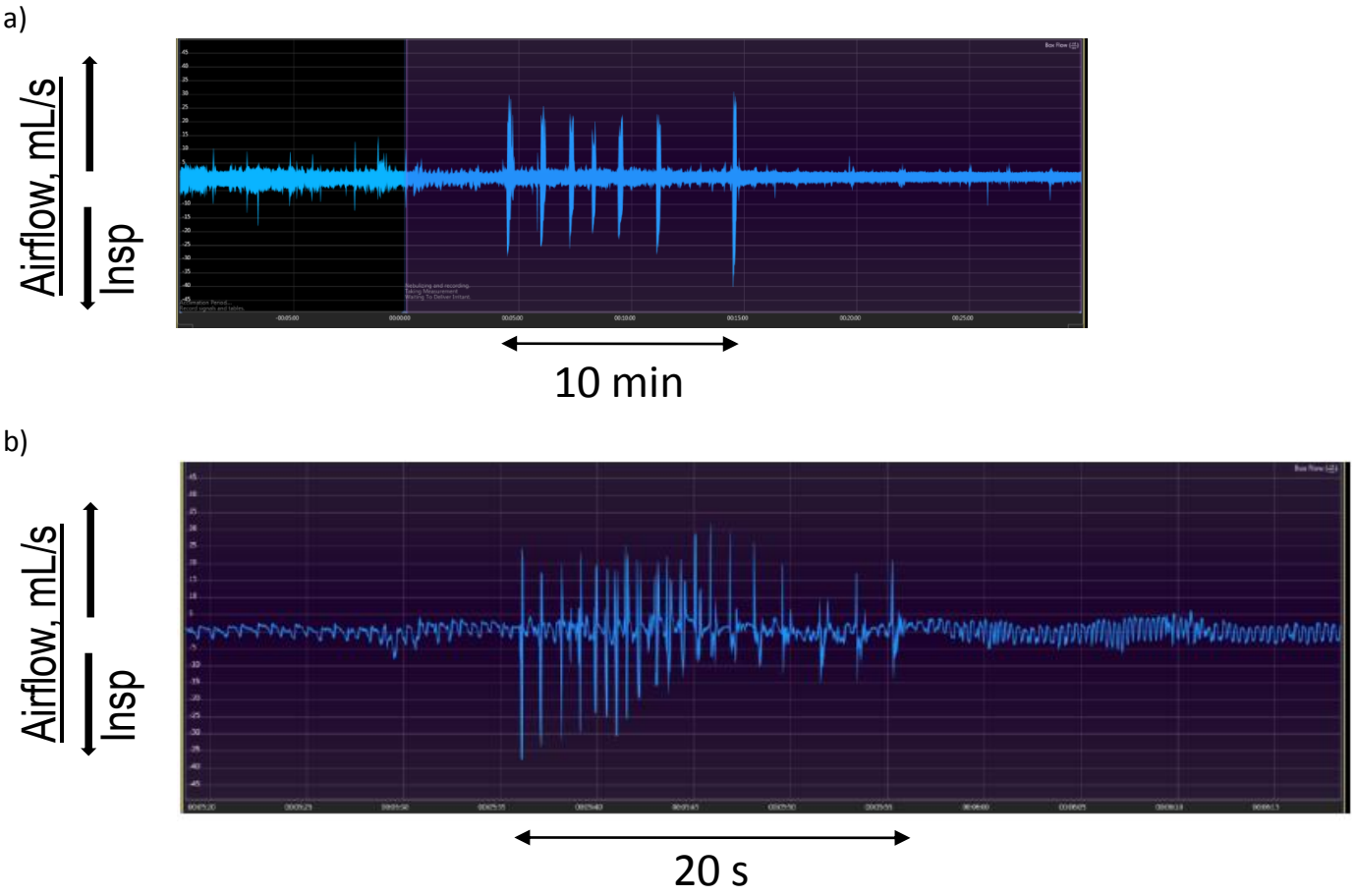


Figure 2. Plethysmograms of 1 guinea pig showing changes in Inspiratory (Insp) and Expiratory (Exp) airflows following exposure to TRE. a) 7 cough "bouts" evoked by a 10-minute exposure to TRE (3.30 µg/kg). b) expanded view of one of the cough bouts with 22 individual coughs enumerated.

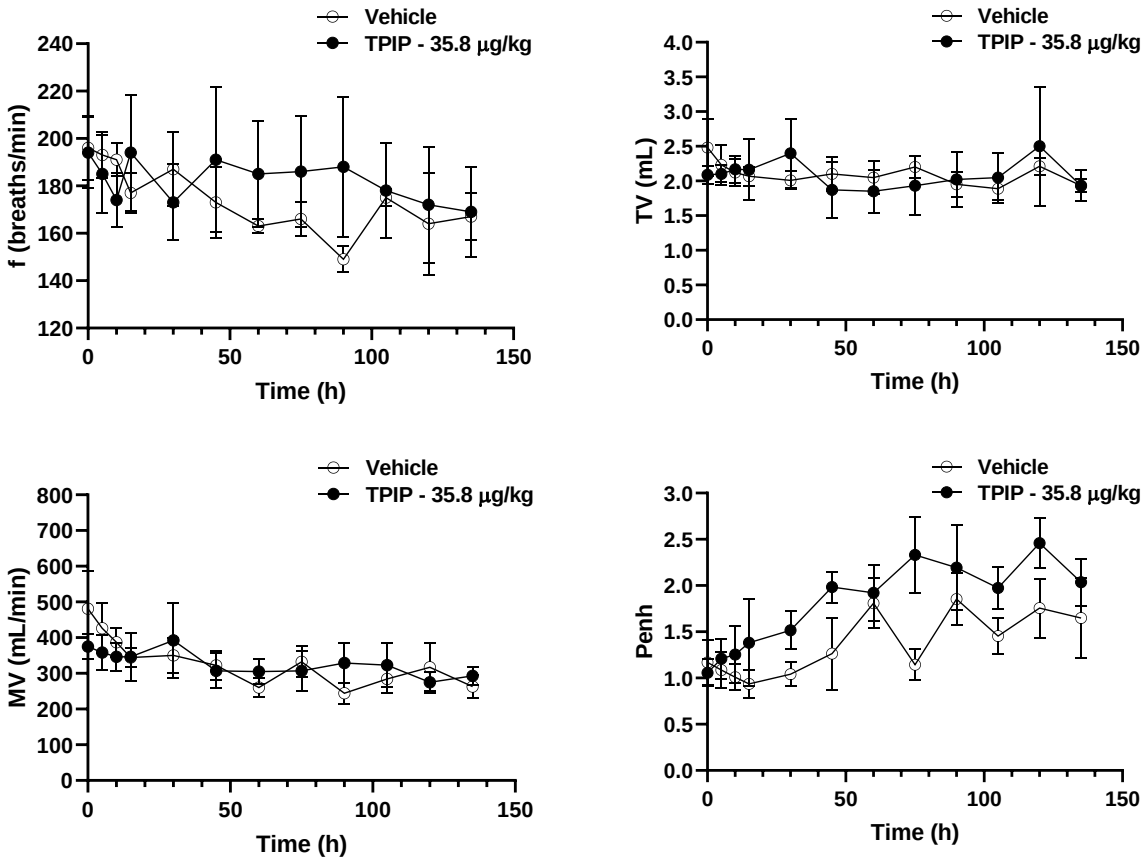
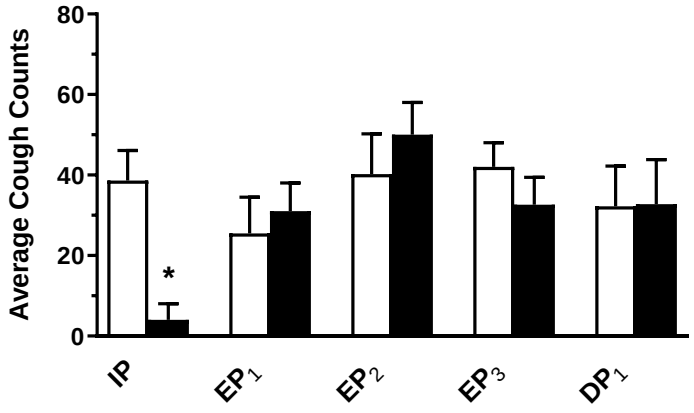


Figure 3. Effect of TPIP and mannitol vehicle on ventilation and Penh in guinea pigs. Data is shown at baseline (time = 0 min), during exposure to TPIP (time = 0-15 min) and following TPIP exposure (time = 15-135 min). TPIP was administered at an inhaled dose of 35.8 µg/kg. Abbreviations: f (respiratory rate), TV (tidal volume), MV (minute ventilation), Penh (enhanced pause). Values are the mean ± SEM (n = 6 for TPIS and n = 4 for mannitol vehicle).

a)



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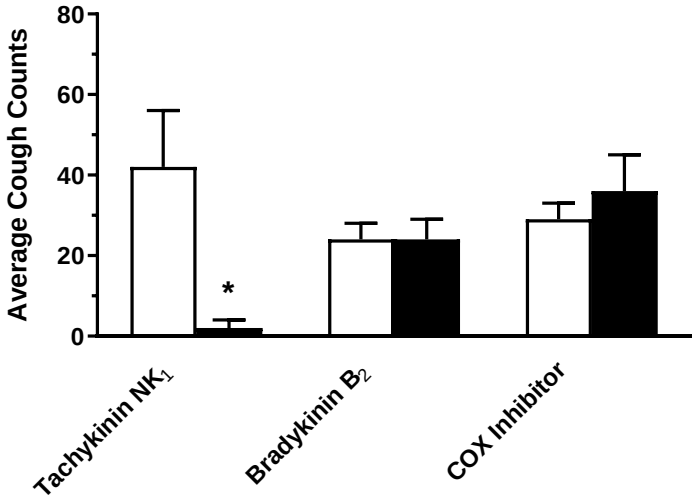
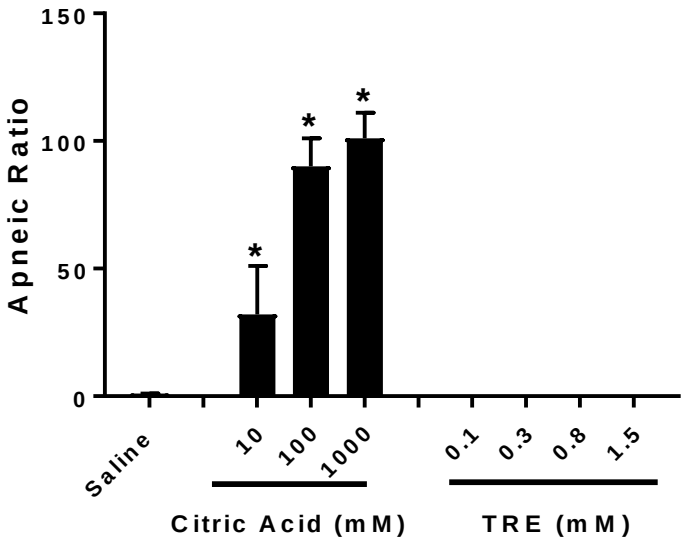


Figure 4. Effects of prostanoid receptor antagonists, a tachykinin NK_1 receptor antagonist, a bradykinin B_2 receptor antagonist and a cyclooxygenase inhibitor on TRE cough in guinea pigs. The compound (black bars) or their respective vehicles (white bars) were administered intraperitoneally (30 min) or by aerosol (10 min) prior to nebulized TRE (3.30 µg/kg). Graphs show the (mean ± SEM) effects against TRE-induced cough of a) the IP receptor antagonist (RO1138452, 10 mg/kg i.p., n = 5), the EP_1 receptor antagonist (ONO-8711, 10 mg/kg i.p., n = 5-6), the EP_2 receptor antagonist (PF-04418948, 5 mg/kg i.p., n = 5), the EP_3 receptor antagonist (L-798106, 10 mg/kg i.p., n = 5-7) and the DP_1 receptor antagonist (BW A868C 10 mg/kg i.p., n = 5) and b) the NK_1 receptor antagonist (CP99994, 10 mg/kg, i.p., n = 5), the bradykinin B_2 receptor antagonist (HOE 140, 1 mg/mL delivered by aerosol, n = 3) and the cyclooxygenase (COX) inhibitor (meclofenamic acid, 1 mg/kg i.p., n = 3). * P <.05 compared to vehicle.

a)



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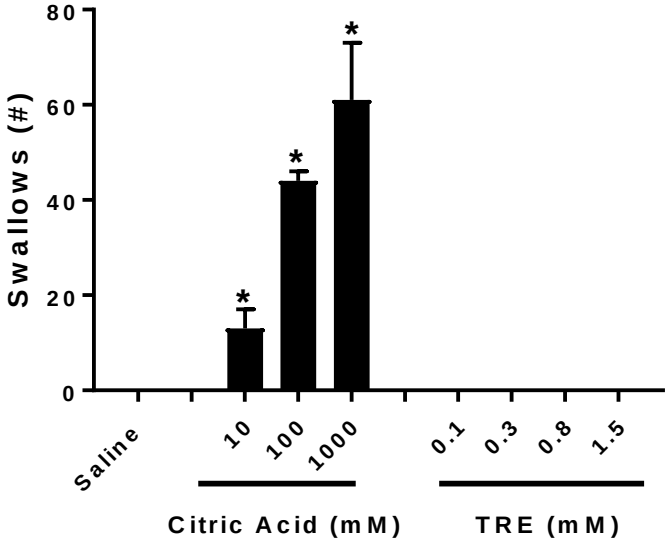
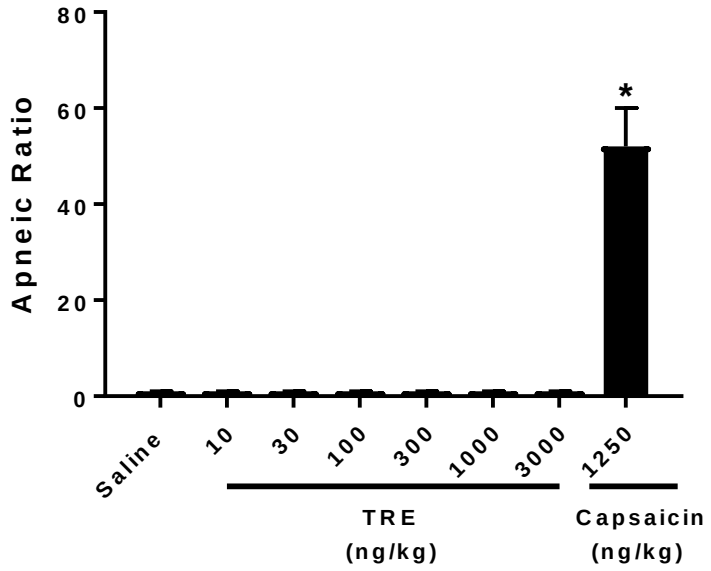


Figure 5. Nebulization of TRE to the isolated laryngeal airway of rats produced no reflex effects, whereas nebulized citric acid produced concentration-dependent a) apnea and b) increased the frequency of swallows. The apneic ratio was calculated as the T_E treatment/T_E baseline where T_E represents the duration of expiration. Values represent the mean ± SEM (n = 5 for saline, n =

5 for citric acid, n = 6 for TRE at 0.1 mM and n = 2 for TRE at 0.3 to 1.5 mM). * *P* <.05 compared to saline.

a)



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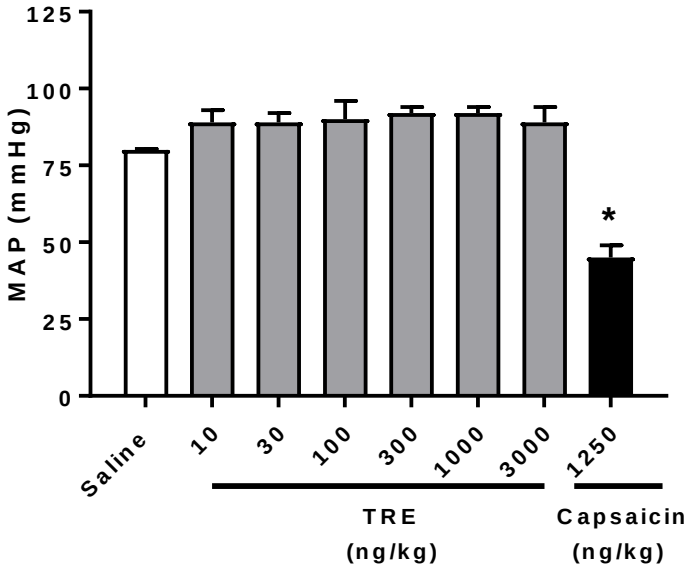


Figure 6. Intravenous TRE produced no reflex effects, whereas intravenous capsaicin produced a) apnea and b) a decrease in mean arterial blood pressure (MAP). The apneic ratio was measured as described in the legend in Figure 5. Values represent the mean ± SEM (n = 3). * P <.05 compared to saline.

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. Insmed 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% O_2/balance N_2) 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 treprostnil 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), EP_1 (ONO-8711), EP_2 (PF-04418948), EP_3 (L-798,106), DP_1 (BWA868C), tachykinin NK_1 (CP99994),and bradykinin B_2 (HOE 140) receptor antagonists and the cyclooxygenase inhibitor, meclofenamic acid. The compounds were administrated 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 (EP_1 antagonist)

The EP_1 antagonist was supplied in methyl acetate which was evaporated to a thin film under flowing nitrogen. The thin film of EP_1 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-□-cyclodextrin (HPBCD) and 40% DMSO (v/v) and vortexed to generate a final concentration of 5 mg/mL of EP_1 antagonist in 50 mg/mL HPBCD, 50% DMSO (v/v), and 0.9% saline (w/v).

III. PF-04418948 (EP_2 antagonist)

The EP_2 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 EP_2 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 (EP_3 antagonist)

The EP_3 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 EP_3 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 (DP_1 antagonist)

The DP_1 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 DP_1 antagonist in 10% ethanol (v/v).

VI. CP 99994 (NK_1 antagonist)

The NK_1 antagonist was dissolved in isotonic saline (0.9% w/v) at a final concentration of 1 mg/mL.

VII. HOE 140 (B_2 antagonist)

The bradykinin B_2 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 µg/kg) and capsaicin (1.25 µg/kg) were sequentially administered into the same rat. Capsaicin at an i.v. dose of 1.25 µg/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 Aeroneb 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 µg/kg) to induce a bronchospasm. Test articles of TRE(1.5 and 3 µg/kg, pulmonary dose) or PBS were delivered directly through the tracheal catheter via an Aeroneb 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).

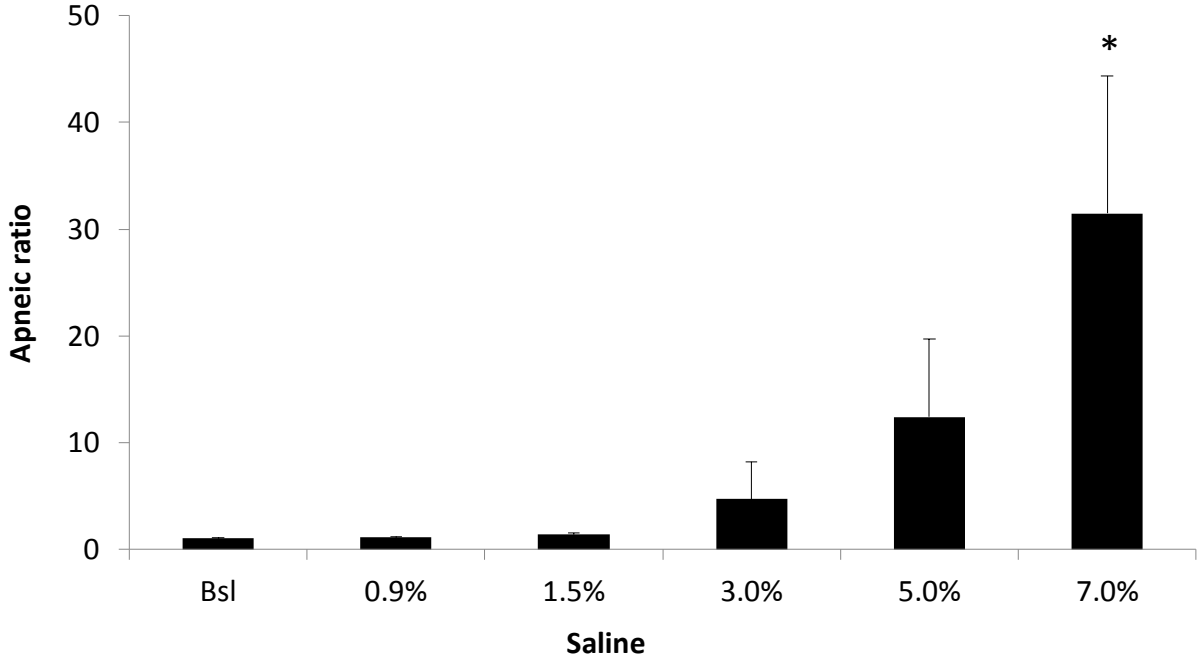
Time = 30-135 min (after test article exposure).

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.

a)



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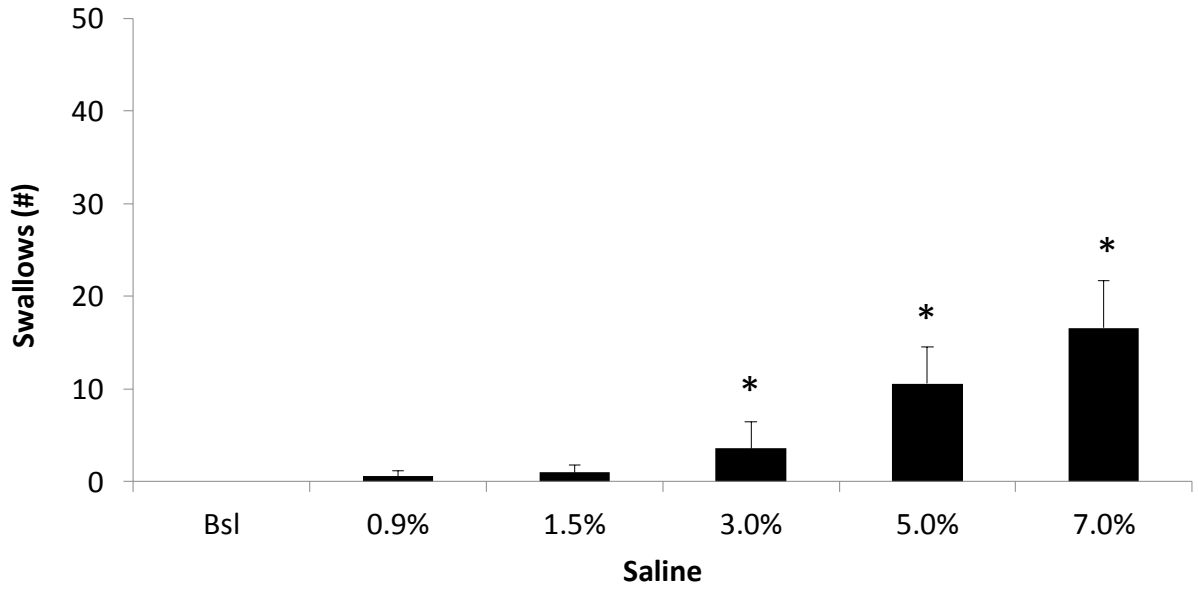


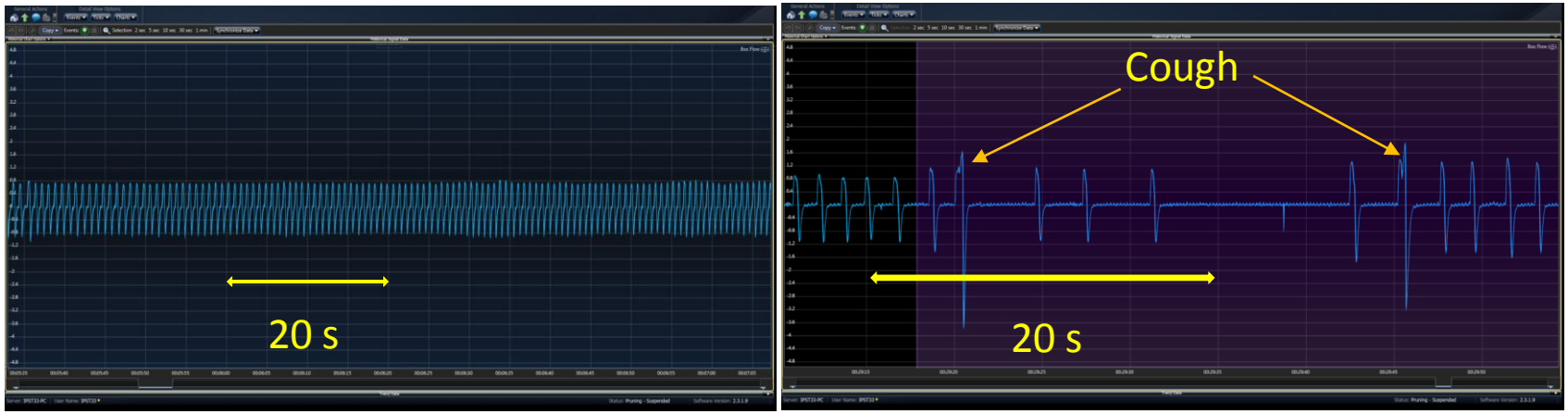
Values are the mean ± SEM (n = 5) for a) the apneic ratio (T_E after nebulization of saline/T_E before nebulization of saline) and b) the frequency of swallows. * $P <$ .05 compared to baseline (Bsl).

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

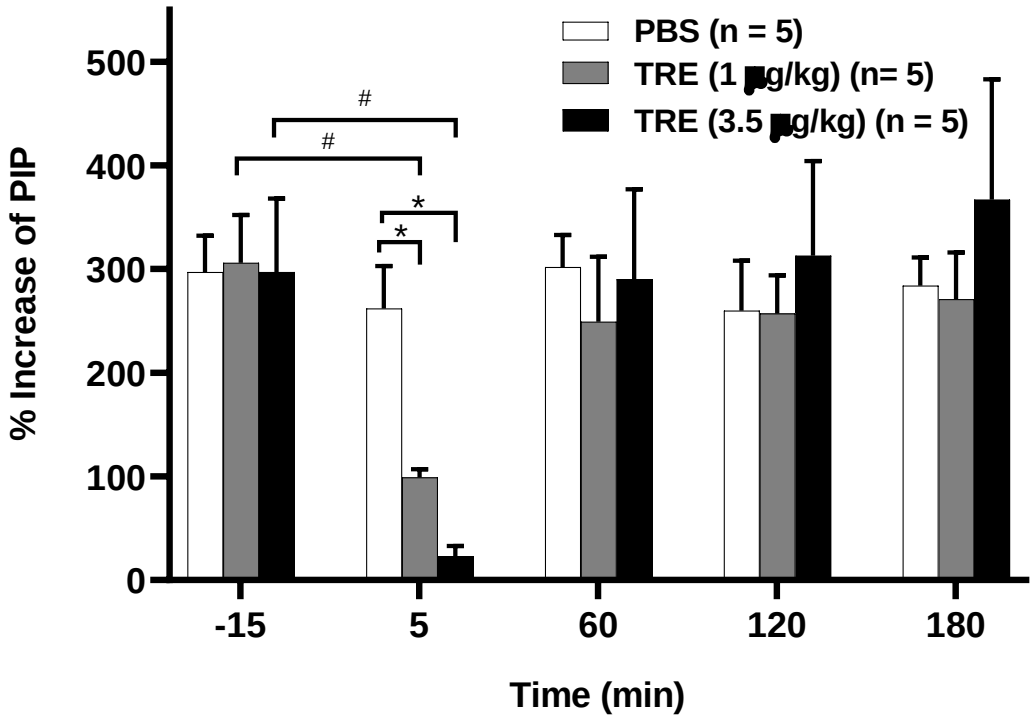
Citric Acid (1M for 20 s)	0.45	6.80	15.11	7
TRE (0.1mM for 3 min)	0.33	0.56	1.70	0

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 µg/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 µg/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_20, 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

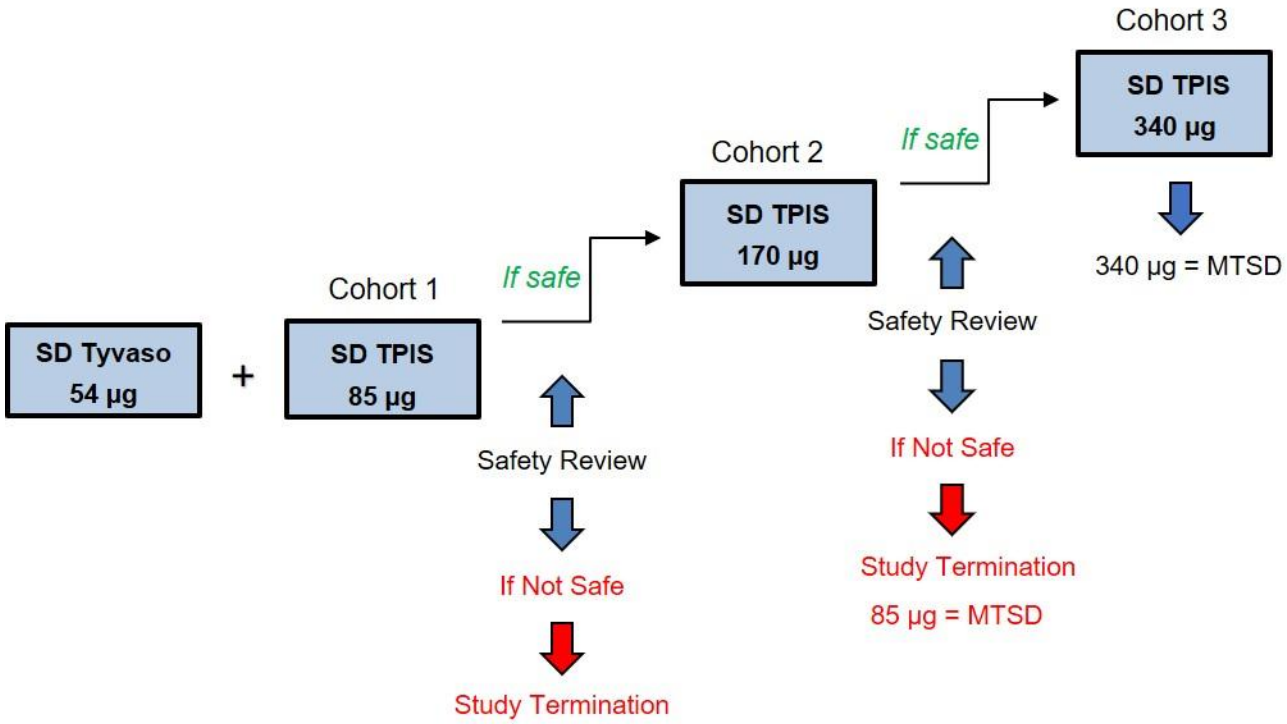
1. Methods

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/m^2 (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 K_2EDTA 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

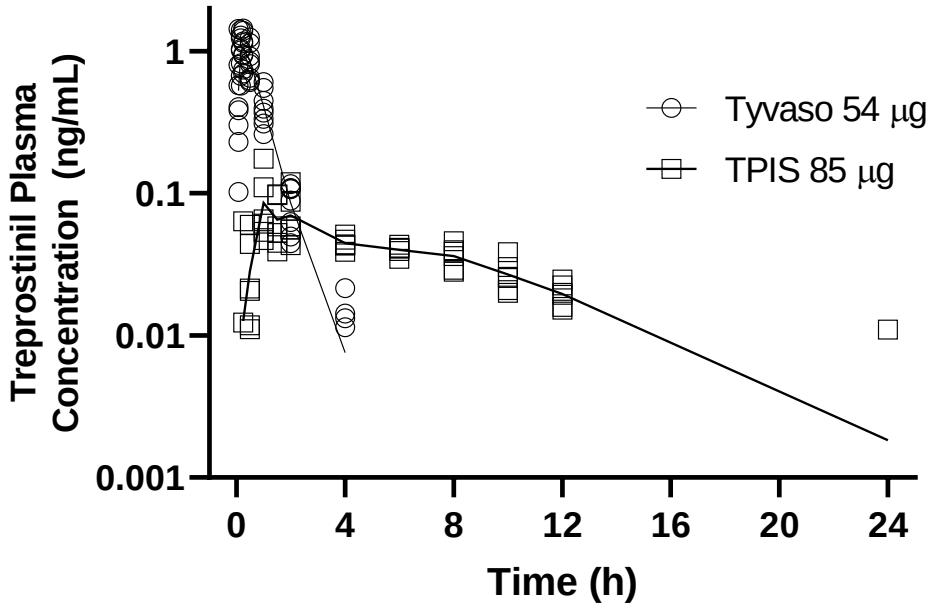
R = Related to treatment; n = Number of subjects dosed with each treatment (or any treatment as applicable); N = Number of subjects with characteristic; % = Calculated using the number of subjects treated with each treatment (or any treatment as applicable) as the denominator (100*n/N).

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 ($AUC_{0\text{-}24}$ 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 $T_{½}$ 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 ρg/mL) were assigned a value of zero.

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

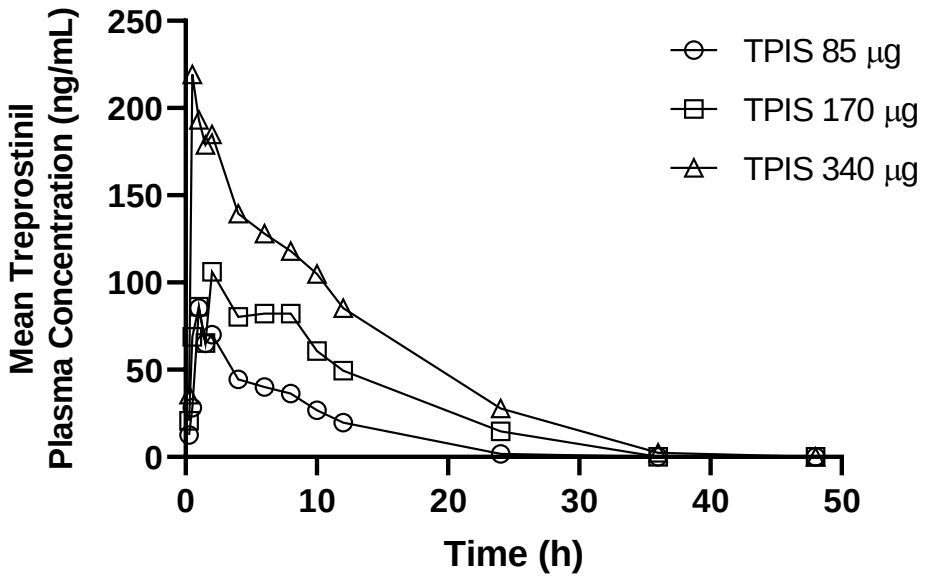
PK Characteristic	Cohort 1		Cohort 2 TPIS 170 µg N = 6	Cohort 3 TPIS 340 µg N = 6
	Tyvaso 54 µg (N = 8)	TPIS 85 µg (N = 6)		
C_{max} (pg/mL)				
Treprostinil	958 (26.5)	89.0 (49.2)	142 (24.9)	318 (33.1)
TP	—	0 (0 – 12.9)[a]	14.7 (0 – 23.0)[a]	34.2 (33.6)
T_{max} (h)[a]				
Treprostinil	0.258 (0.183 – 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)
AUC_{0-6} (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)
AUC_{0-24} (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)

AUC_{0-6}, area under the concentration-time curve from 0 to 6 hours; AUC_{0-24}, 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 $T_{½}$ (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 ρg/mL) were assigned a value of zero.

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