Pulmonary Pharmacology & Therapeutics 49 (2018) 104–111

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

# Inhaled hexadecyl-treprostinil provides pulmonary vasodilator activity at significantly lower plasma concentrations than infused treprostinil

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ARTICLE INFO

*Keywords:*
Pulmonary vasodilation
Treprostinil
Hexadecyl-treprostinil prodrug
Rats
Dogs
Local lung effect

ABSTRACT

INS1009 is a long acting pulmonary vasodilator prodrug of treprostinil (TRE) that is formulated in a lipid nanoparticle for inhaled delivery by nebulization. This study examined the ability of INS1009 to inhibit vasoconstriction in the pulmonary vasculature of rats and dogs and the extent to which local activity within the lung contributes to its activity. Rats received a single dose of INS1009 by nose-only inhalation or were given a continuous intravenous (i.v.) infusion of TRE, followed by an i.v. challenge of the thromboxane mimetic pulmonary vasoconstrictor U46619 and the increase in pulmonary arterial pressure (PAP) was measured. In beagle dogs, INS1009 was given by inhalation via face mask and TRE was given by continuous i.v. infusion; vasoconstriction was then induced by inhaled hypoxia with reduction of $FIO_2$ to 0.10. Changes in the dog's right ventricular pulse pressure (RVPP) were measured using implanted telemetry probes. Blood samples were collected in rats and dogs immediately after the challenge to measure the plasma TRE concentration. Exposure of rats to inhaled INS1009 (0.5, 3.0 and 20.9 μg/kg) inhibited the U46619-induced increase in PAP at all doses up to 6 h with statistically significant inhibition up to 24 h with the pooled dose-response data. The concentration of TRE in the plasma at which PAP was reduced by 50% was approximately 60-fold lower for INS1009 ($EC_{50}$ = 0.08 ng/mL) as compared to i.v. TRE ($EC_{50}$ = 4.9 ng/mL). In dogs, INS1009 (2.7–80.9 μg/kg) inhibited the hypoxia-induced increase in RVPP at all doses up to 6 h with activity once again observed with the pooled dose-response of 10 μg/kg and higher at 24 h. The concentration of TRE in the plasma at which RVPP was reduced by 50% was approximately 550-fold lower for INS1009 ($EC_{50}$ = 0.0075 ng/mL) as compared to i.v. TRE ($EC_{50}$ = 4.1 ng/mL). These studies, in two species and by two different pulmonary vasoconstrictor challenges, demonstrate that inhaled INS1009 not only has long-acting vasodilatory effects but also that the local activity within the lung contributes to this response. Therefore, INS1009 may offer the opportunity to effect pulmonary vasodilation for long periods but with substantially lower systemic exposure than infused TRE.

## 1. Introduction

Treprostinil (TRE) is a prostacyclin agonist that is used for the treatment of pulmonary arterial hypertension (PAH) [1–3]. TRE is available for administration by the parenteral (Remodulin®), oral (Orenitram®) and inhaled (Tyvaso®) routes [4–6]. While Remodulin is continuously infused over a 24-h period, there are concerns with its administration including line infection (intravenous (i.v.) infusion) and infusion site pain (subcutaneous infusion) and adverse effects (AEs) are notable, including headache, nausea, jaw pain and vasodilation [4,7]. Orenitram provides convenient administration but requires repeated daily dosing (recommended b.i.d or t.i.d.) and patients still experience AEs associated with systemic exposure [5,8]. In addition to many of the same AEs, Tyvaso is reported to evoke cough, and dose administration is burdensome at 4 times daily [6,9].

There are inhaled antibiotic drug formulations that provide effective activity locally within the lung that also demonstrate low systemic exposure (reduced toxicity) due primarily to their slow-release design [10]. While inhaled TRE (Tyvaso) is locally administered to the lung, it rapidly diffuses into the systemic circulation [9]. We reasoned that an inhaled slow-release TRE formulation would not only provide prolonged activity with reduced dosing frequency but also yield lower peak

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https://doi.org/10.1016/j.pupt.2018.02.002
Received 22 September 2017; Received in revised form 22 January 2018; Accepted 5 February 2018
Available online 05 February 2018
1094-5539/ 

plasma levels and the associated side effects with systemic TRE exposure. In order to achieve these product attributes, an ester-linked hexadecyl-treprostinil prodrug (C16TR) formulated in a lipid nanoparticle was developed for inhaled delivery by nebulization [11,12]. This formulation (INS1009) has extended pulmonary vasodilator activity in rats, has low plasma TRE $C_{max}$ and maintains sustained levels of C16TR and TRE in the lungs and TRE in the plasma in rats and dogs over 24 h after inhalation [11]. In a phase 1 study in healthy volunteers, inhaled INS1009 (85 μg) had a low plasma TRE $C_{max}$ and sustained levels of TRE in the plasma over 12 h compared to inhaled TRE (54 μg) [13,14]. Furthermore, INS1009 has fewer side effects in guinea pigs and dogs compared to inhaled TRE [11]. On the basis of these studies, it is proposed that INS1009 would offer sustained pulmonary vasodilation greater than inhaled TRE. However direct measurements of pulmonary vasodilation with inhaled INS1009 have only been performed in anesthetized, hypoxia challenged rats with activity measured up to the technical limit of this preparation which is 3 h [11]. Therefore, alternate test systems are required if pulmonary vasodilation is to be assessed for longer periods of time.

In a previous study in sheep, continuously inhaled TRE was found to be more efficacious than continuously i.v. infused TRE as a pulmonary vasodilator with reduced systemic side effects [15]. It was proposed that these positive attributes of inhaled TRE were due to a "localized" lung effect. Although continuous inhalation in patients is not practical these results have important implications for the activity of INS1009 since with a single inhalation of INS1009, TRE is supplied to the lung over a prolonged period as C16TR prodrug converts to active TRE [11]. A "localized" lung effect would occur when pulmonary vasodilation is observed after inhalation but plasma levels of TRE are below the levels necessary for injected (systemic) TRE to achieve pulmonary vasodilation.

The goal of the present studies was to investigate the duration of activity of INS1009 over 24 h and to determine if local lung effects contribute to its pulmonary vasodilator response. Experiments were performed in rats and dogs with inhaled INS1009 given by inhalation followed by the challenge with the pulmonary vasoconstrictor U46619 for rats [16] or with inhaled hypoxia for dogs [17,18] at different times after inhalation over 24 h. To determine if local activity in the lung contributes to its effects, the inhibition of pulmonary vasoconstriction by INS1009 was compared to the effects of i.v. TRE as a function of the plasma TRE concentration.

## 2. Materials & methods

### 2.1. Materials

INS1009, an inhaled formulation of the prodrug hexadecyl-treprostinil (C16TR) in a lipid nanoparticle was produced at Insmed Incorporated (Bridgewater, NJ, USA) using a proprietary process. C16TR was produced by Chirogate International Incorporated (Taiwan). The components of INS1009 are C16TR, squalane and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(poly-ethylene glycol)-2000] (DSPE-PEG2000) in a molar ratio of 45/45/10. Each of these components were dissolved in ethanol and injected into perpendicular impinging streams with phosphate buffered saline (PBS) using a mixing cross configuration. The lipid nanoparticles formed by this process were suspended in PBS and exhibited mean diameters ranging from 100 to 150 nm as measured by dynamic light scattering. Additional details of the TRE prodrug synthesis and incorporation into a lipid nanoparticle are described in detail elsewhere [12].

### 2.2. Animals

Male Wistar Han rats (Envigo CRS Incorporated, Lachine, Quebec, Canada) of weight range 200–250 g were housed three per cage and received food and water ad libitum. All experimental procedures were performed in accordance with the Canadian Council on Animal Care (CCAC) guidelines. Two male beagle dogs (Marshall Farms, North Rose, NY, USA) approximately 12 months of age, underwent surgery for the placement of epicardial electrocardiogram (ECG) leads and catheterization of the right ventricle and abdominal aorta. The Advanced Research Technology software package from Data Sciences International (DSI, Incorporated, St. Paul, MN, USA) was used to collect telemetered right ventricular pressures (systolic, diastolic, mean, pulse), systemic blood pressures (systolic blood pressure, diastolic blood pressure, systemic mean arterial blood pressure [MAP]) and heart rate (HR) and the IOX software package (EMKA Technologies, Paris, France) was used to collect telemetered respiratory impedance plethysmography data (respiratory rate, tidal volume and minute volume). All experimental procedures performed in telemetered dogs were performed with prior approval of the Institutional Animal Care and Use Committee (IACUC) at Envigo, CRS (East Millstone, NJ, USA) which is a facility accredited by the Association for Assessment and Accreditation of Laboratory and Animal Care (AAALAC).

### 2.3. Experiments in rats

Exposure to inhaled INS1009 or PBS was performed using a 12-port rodent nose-only inhalation tower (CH Technologies, Westwood, NJ, USA). Aerosols were generated using an Aeroneb® Pro nebulizer (Aerogen, Galway, Ireland) that was connected to an air source and delivered through the tower at a flow rate of 6 L/min. After exposure of the rats to either INS1009 or PBS, they were anesthetized with 3% isoflurane-oxygen to implant a femoral venous catheter followed by transition to an i.v. ketamine/xylazine mixture (10 and 1 mg/mL, respectively, infusion rate of 0.02 mL/min) for the duration of the study. The rats were prepared with tracheal, pulmonary arterial and femoral arterial blood pressure catheters. The mean pulmonary arterial blood pressure (PAP) and MAP were measured. The mean PAP was derived by adding one third of the difference between the systolic and diastolic pulmonary arterial pressures to the diastolic pulmonary pressure. A pulse oximeter was placed on the paw to measure arterial oxygen saturation ($SaO_2$) and HR. The rats were artificially ventilated throughout the study. Cardiovascular parameters were measured every 5 ms before and after i.v. infusion of U46619 (5 μg/kg) which was infused over a 40 s period. The response to U46619 challenge was calculated as % increase in mean PAP taken from baseline values obtained immediately before U46619 to peak values obtained after the U46619 infusion. At the end of the study, the rats were euthanized, the lungs were harvested and a blood sample was collected to measure the concentrations of C16TR and TRE (see Bioanalytical measurements). Experiments were also performed in rats treated with different concentrations of i.v. infused TRE (infusion rate of 0.05 mL/kg/hr) to achieve plasma TRE concentrations between 0.1 and 100 ng/mL. Once the target TRE concentration was achieved, rats were challenged with U46619, the PAP response measured and a blood sample was obtained to measure the plasma TRE concentration.

For inhalation studies, the experiments involved exposure of rats to either PBS (n = 12) or INS1009 (n = 4–6) followed by challenge with U46619 at times of 1 h (immediately post dose), 6 h, 12 h and 24 h. In pilot studies, U46619 challenge gave consistent increases in PAP performed at different times over a 24 h period. Therefore, to minimize the number of PBS controls required for these studies, 12 rats were randomly assigned to a U46619 challenge at different times over 24 h time. Values for the PBS control were represented as a single average value and used to compare values for each dose of INS1009 using a 1-way ANOVA with Tukey's range test for both the individual or pooled data that was generated from the dose-response studies.

### 2.4. Experiments in dogs

Eight separate experiments were performed with INS1009 in which the dogs were exposed to INS1009 or PBS for 15–30 min by inhalation