A 14-Day Assessment of the Tolerability and Pharmacokinetics of a Nanoparticle Formulation of Hexadecyl-Treprostinil, a Long-Acting Pulmonary Vasodilator, in Rats

Poster #: P496

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INTRODUCTION

- Inhaled treprostinil (TRE) is a pulmonary vasodilator developed for the treatment of pulmonary arterial hypertension (PAH).[1]
- Although inhaled TRE has met with clinical success, it must be administered 4 times per day to maintain bioactivity over 24 hours and is associated with a number of adverse events (AEs), the most prevalent of which is cough.[1]
- Previous studies in both rats and dogs demonstrate that single dose inhalation with a nanoparticle formulation INS1009 of the treprostinil prodrug, hexadecyl-treprostinil (C16TR) is well tolerated, produces long-acting pulmonary vasodilation and sustained levels of treprostinil (TRE) in the plasma and C16TR in the lungs.
- In this study in rats, we assessed the PK of and AEs associated with INS1009, a lipid nanoparticle formulation of TRE prodrug hexadecyl treprostinil (C16TR), administered at 4 different doses over a period of up to 14 days.

METHODS

- Male Sprague Dawley rats were placed in restraining tubes and exposed to nebulized drugs via the 12-port Jaeger-NYU Nose-Only Directed-Flow Inhalation Exposure System (CH Technologies, Westwood, NJ) **(Figure 1)**. An air flow carrying aerosol generated by nebulizer was provided to the system tower at a rate of 6 L/min.
- INS1009 was administered using Aeroneb® Pro nebulizer (Aerogen, Galway, Ireland) to deliver estimated pulmonary doses of 1 µg/kg, 3 µg/kg, 10 µg/kg, and 30 µg/kg. Rats received once-daily doses for 1 day, 7 days, or 14 days, depending on the assigned group. A 6 mL volume of INS1009 at varying concentrations of C16TR (0.1, 0.3, 1, and 3 mM) was nebulized at a rate of approximately 0.2 mL/min.
- PK analysis: Blood samples were taken at selected times after nebulization over a 24-hour period, and lung samples were collected 24 hours after the last dose. TRE levels and C16TR concentrations in blood plasma and lung tissue were measured by high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS).
- Respiratory tissue: Lungs, tracheae, and larynges were also collected after the last dose for histologic examination.
- Right lobe was sliced (5-µm thick) and stained with Oil-Red-O (O-R-O) for lipid concentration analysis. The middle left lobe, trachea, and larynx were sliced and stained with hematoxylin and eosin (H&E) for evaluation of tissue damage or with Periodic acid–Schiff (PAS) for identification of goblet cells in the airway epithelium.

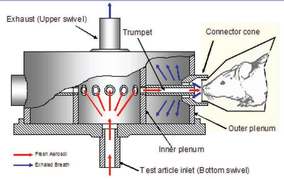


Figure 1. Jaeger-NYU Nose-Only Directed-Flow Inhalation Exposure System (CH Technologies, Westwood, NJ) used to expose rats to nebulized INS1009 aerosol.
Reproduced from CH Technologies (USA), Inc. with permission.

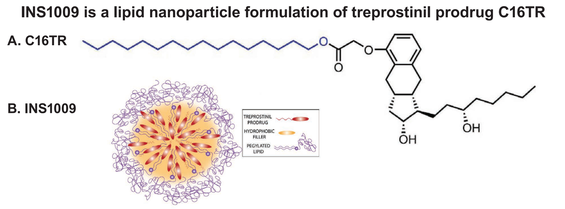


Figure 2. *(A)* Chemical structure of treprostinil prodrug C16TR . *(B)* Schematic representation of INS1009, a C16TR lipid nanoparticle (~100 nm in diameter). Hydrophobic filler is squalane. Pegylated lipid is DSPE-PEG2000.

RESULTS

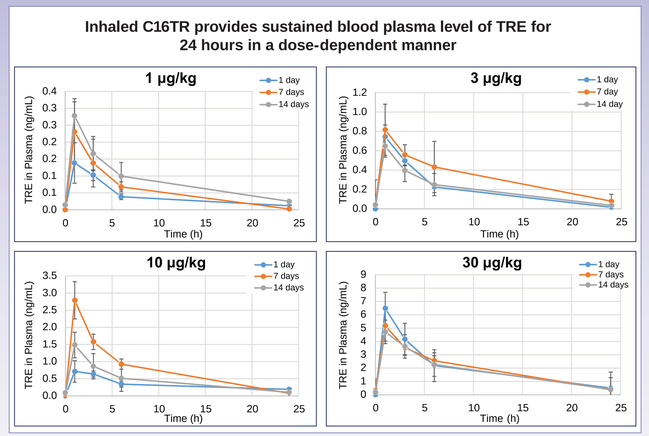
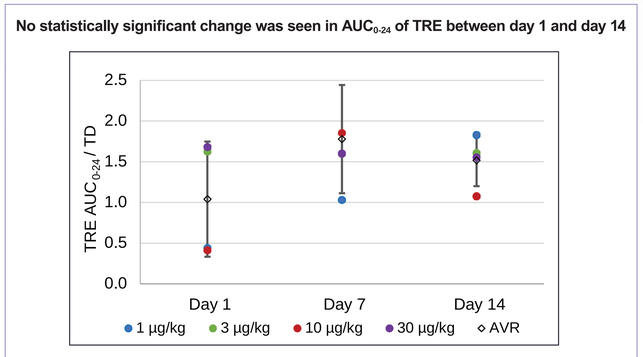


Figure 3. Blood plasma TRE in rats after dosing with nebulized INS1009 for 1, 7, or 14 days. The targeted pulmonary doses are shown. Values are the mean ± standard deviation of 4 rats.

Table 1. Pharmacokinetic Parameters of TRE in Blood Plasma on Days 1, 7, and 14 in Rats After Dosing with Inhaled C16TR

Targeted Lung Dose	Group	TRE IBD (ng/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24}$ (h*ng/mL)	AUC/TD
1 µg/kg	Day 1	0	0.14	0.44	0.44
	Day 7	0	0.23	1.03	1.03
	Day 14	0.015	0.28	1.83	1.83
3 µg/kg	Day 1	0	0.75	4.88	1.63
	Day 7	0.042	0.82	7.89	2.63
	Day 14	0.069	0.63	4.82	1.61
10 µg/kg	Day 1	0	0.72	4.15	0.42
	Day 7	0.042	2.79	18.5	1.85
	Day 14	0.093	1.48	10.8	1.08
30 µg/kg	Day 1	0	6.48	50.4	1.68
	Day 7	0.297	5.18	48.0	1.60
	Day 14	0.190	4.71	46.6	1.55

$AUC_{0\text{-}24}$, area under the curve during 24 hours; C_{max}, maximum plasma concentration; IBD, immediately before dosing; TD, targeted dose; T_{max}, time to maximum plasma concentration.



$AUC_{0\text{-}24}$, area under the curve during 24 hours; AVR, average; TD , targeted dose.

Figure 4. $AUC_{0\text{-}24}$ of TRE normalized for the C16TR targeted dose on days 1, 7, and 14 in rats. Average of all 4 doses and standard deviations are shown.

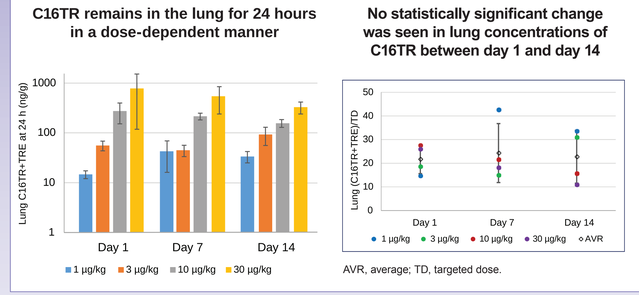


Figure 5. Combined concentration of C16TR and TRE in the lung of rats 24 hours after the last dose of inhaled INS1009 in each group. The concentration is calculated as equivalent of C16TR, based on molecular weight.

AVR, average; TD, targeted dose.

Figure 6. Combined concentration of C16TR and TRE in the lung of rats 24 hours after the last dose normalized for the C16TR targeted dose on days 1, 7, and 14. Average of all 4 doses and standard deviations are shown.

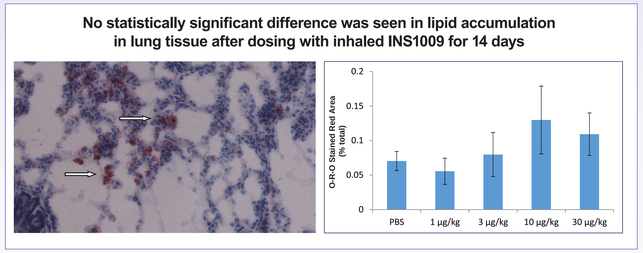


Figure 7. Representative photomicrograph of lung preparations of rats treated with 10 µg/kg of inhaled INS1009 for 14 days. Preparation was stained with Oil-Red-O (O-R-O). Magnification 200x. Arrows indicate abundance of medium-sized lipid clusters in the tissue.

Figure 8. Lipid concentration determined by Oil-Red-O (O-R-O) staining from lung tissue from rats treated with inhaled INS1009 after 14 days of dosing. Values are the mean ± standard error of the mean of 8 (PBS, phosphate-buffered saline) or 4 (INS1009) rats.

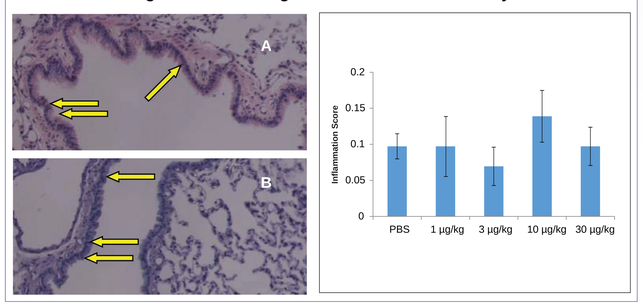


Figure 9. Representative photomicrograph of lung preparations of rats treated with 10 µg/kg of inhaled INS1009 for 14 days. Preparations were stained with hematoxylin and eosin (H&E) *(A)* or with Periodic acid−Schiff (PAS) *(B).* Arrows indicate the goblet cells.

Figure 10. Inflammation scores of lung tissue from rats treated with inhaled INS1009 after 14 days of dosing. Scoring was based on the presence of neutrophils, macrophages, and other inflammatory cells, as well as signs of peribronchial and perivascular inflammations. Values are the mean ± standard error of the mean of 8 (PBS) or 4 (INS1009) rats.

No significant change was seen in body weight or lung weight in rats after dosing with inhaled INS1009 for 14 days. Macroscopic changes of the lungs were mild when compared with rats treated with inhaled TRE

Table 2. Body Weight and Lung Weight in Rats Treated With Inhaled INS1009 and Inhaled Treprostinil After 14 Days of Dosing

Formulation	Dose (µg/kg)	BW (g)	Lung weight (g)	Lung/BW
INS1009	1	383.5	1.26	0.33
INS1009	3	404.0	1.34	0.33
INS1009	10	414.8	1.47	0.36
INS1009	30	403.8	1.50	0.37
TRE	1*	412.3	1.36	0.33
TRE	3*	400.8	1.38	0.34

BW, body weight. Each data point is the mean of 4 rats.

*Dose for TRE is shown as C16TR equivalent. Macroscopic observation of lungs revealed no abnormalities in INS1009-treated rats at 1 µg/kg and 3 µg/kg but found hemorrhagic spots and edema in the TRE-treated rats. Dark spots were found on the lung surface in INS1009-treated rats at 10 µg/kg and 30 µg/kg doses.

CONCLUSIONS

- No difference was observed in the PK profile between day 1 and day 14 of dosing with inhaled INS1009 for both TRE level in the plasma and C16TR concentration in the lungs.
- Inhaled INS1009 (1-30 µg/kg C16TR) was well tolerated, with no major changes in body weight and lung weight after dosing for 14 consecutive days.
- No tolerability issues or altered PK are expected in studies involving repeated dosing with inhaled INS1009.

REFERENCE

- Tyvaso (treprostinil) inhalation solution, for oral inhalation only [package insert]. United Therapeutics Corp. (Research Triangle Park, NC). August 2014.

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