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Structure-Dependent Pharmacokinetic Profile of Alkyl Ester Treprostinil Prodrugs Administered via Metered Dose Inhaler to Rats

Adam Plaunt, Richard W. Chapman, Michel R. Corboz, Zhili Li, Walter R. Perkins, Vladimir Malinin

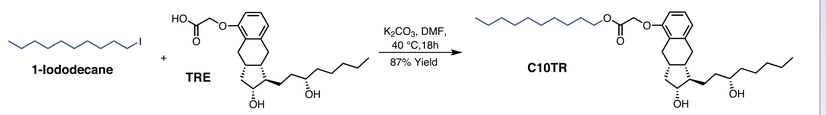
Insmed Incorporated, Bridgewater, NJ, USA.

INTRODUCTION

- Treprostinil (TRE), a prostacyclin pulmonary vasodilator, has been approved for the treatment of pulmonary arterial hypertension (PAH) and is available as a solution for inhalation (Tyvaso®), an oral tablet (Orenitram®), and an injection (Remodulin®).[1] However, because of a short elimination half-life (≈ 4 hours), inhalation treatment of PAH with TRE requires frequent dosing to sustain a long-acting pulmonary vasodilation.[1]
- In addition, inhalation of TRE is associated with local and systemic adverse events, including cough, headache, and throat irritation.[1]
- To extend the therapeutic effect, we designed alkyl ester prodrugs of treprostinil (TPD) that convert slowly to TRE and examined how the length of the alkyl ester chain affects pharmacokinetic (PK) profiles for TPDs delivered via metered dose inhaler (MDI).[2]

METHODS

- TPDs were prepared using standard synthetic chemistry techniques. A representative synthesis scheme is shown below.
  - TPDs include hexadecyl treprostinil (C16TR), tetradecyl treprostinil (C14TR), dodecyl treprostinil (C12TR), undecyl treprostinil (C11TR), decyl treprostinil (C10TR), 5-nonanyl treprostinil (5C9TR), and octyl treprostinil (C8TR).



C10TR, decyl treprostinil; DMF, N,N-dimethylformamide; K_2CO_3, potassium carbonate; TRE, treprostinil.

Scheme 1. Representative synthesis scheme for preparing TPDs.

- MDI canisters containing TPD (1 mg/mL), propellant (either HFA-134a or HFA-227ea), and ethyl alcohol (EtOH) cosolvent were prepared using a 2-stage MDI filling process. Qualitative solubility and stability tests were performed to optimize formulation composition. Final canister compositions were as follows:

TPD	TRE	C8TR	5C9TR	C10TR	C11TR	C12TR	C14TR	C16TR
EtOH (wt %)	10	5	5	5	5	5	7	13
Propellant	HFA-227	HFA-134	HFA-134	HFA-134	HFA-134	HFA-134	HFA-134	HFA-227

5C9TR, 5-nonanyl treprostinil; C8TR, octyl treprostinil; C10TR, decyl treprostinil; C11TR, undecyl treprostinil; C12TR, dodecyl treprostinil; C14TR, tetradecyl treprostinil; C16TR, hexadecyl treprostinil; EtOH, ethyl alcohol; TPD, treprostinil prodrug; TRE, treprostinil.

- Rats were exposed to aerosolized drugs using the Nose-Only Inhalation ADG type chamber (ADG Developments Ltd, Hitchin, UK) equipped with the automatic MDI actuation system (Envigo Life Sciences, East Millstone, NJ, USA).
- Bioanalytical analysis of the samples from in vivo studies was conducted using tandem liquid chromatography-mass spectrometry.
- Single-Dose PK Studies: Male Harlan Wistar Han rats (200-250 g) were exposed to an aerosolized TPD at a targeted dose of either 10, 30, 100, or 300 µg/kg. After exposure, 4 rats in each treatment group were euthanized by exsanguination and blood and lung tissue samples were collected at different times after exposure to the drugs.
- Repeat-Dose PK Studies: Male Harlan Wistar Han rats (200-250 g) were exposed to an aerosolized TPD for 5 days twice daily (bid) at a targeted dose of either 0 µg/kg (groups A and D), 100 µg/kg (groups B and E), or 300 µg/kg (groups C and F). Rats were euthanized (4 rats per group at each time point), and blood and lung tissue samples were collected at different times after exposure to the drugs.

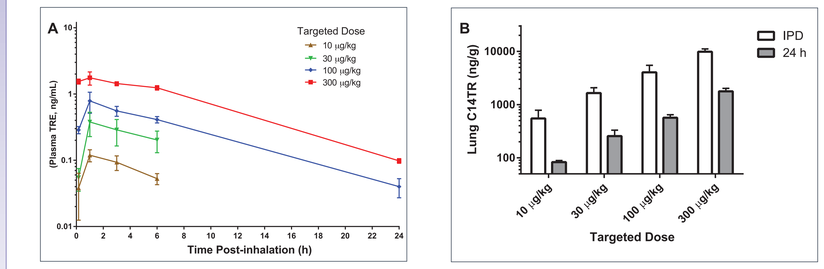
Group	Test Article[a]	Targeted Dose (µg/kg)	Dose Duration (min)	Number of Animals
A	Vehicle	0	36	9
B	C10TR	100	12	51
C	C10TR	300	36	51
D	Vehicle	0	36	9
E	C12TR	100	12	51
F	C12TR	300	36	51

C10TR, decyl treprostinil; C12TR, dodecyl treprostinil.
[a]All canisters were prepared in HFA-134a with 5% EtOH wt/wt.

RESULTS

Single-Dose PK Studies

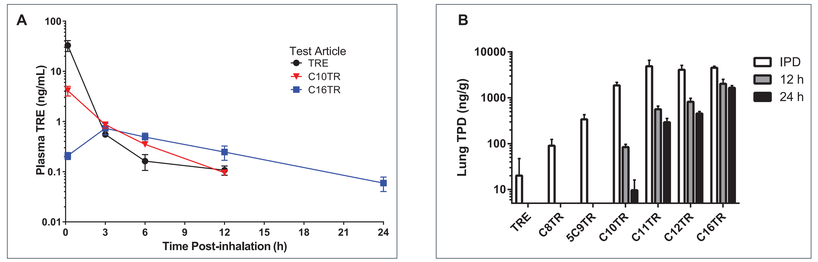
C14TR delivered by MDI provides sustained levels of plasma TRE for up to 24 h. An average of 17% C14TR remained in the lung tissue 24 h after dosing, indicating slow clearance from lung.



C14TR, tetradecyl treprostinil; IPD, immediately post-dose; MDI, metered dose inhaler; TRE, treprostinil.

Figure 1. Plasma TRE (A) and lung C14TR (B) levels after metered dose inhaler administration of C14TR at targeted doses of 10, 30, 100, and 300 µg/kg.

Alkyl chain length of TPDs affected both TRE plasma PK and TPD lung clearance. Longer-chain TPDs such as C16TR exhibit delayed plasma kinetics (longer T_{max}, lower C_{max}) and prolonged clearance from the lungs relative to both TRE and shorter chain TPDs such as C10TR.



5C9TR, 5-nonanyl treprostinil; C8TR, octyl treprostinil; C10TR, decyl treprostinil; C11TR, undecyl treprostinil; C12TR, dodecyl treprostinil; C16TR, hexadecyl treprostinil; C_{max}, maximum concentration; IPD, immediately post-dose; PK, pharmacokinetic; T_{max}, time at which the maximum plasma TRE concentration (C_{max}) is observed; TPD, treprostinil prodrug; TRE, treprostinil.

Figure 2. Plasma TRE (A) and lung TPD (B) levels after metered dose inhaler administration of various TPDs at a targeted dose of 100 µg/kg.

Table 1. Alkyl Chain Length of TPDs Affects Both TRE Plasma PK and TPD Lung Clearance

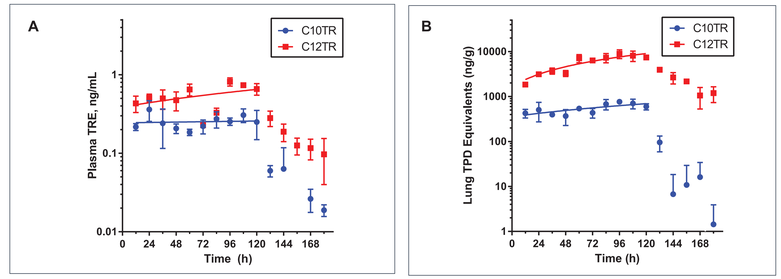
Compound	Plasma C_{max} (ng/mL)	Plasma T_{max} (h)	Plasma AUC (ng*h/mL)	Residual TPD in lung at 12 h (%)	Residual TPD in lung at 24 h (%)
TRE	32.85	0.17	18.74	0	0
C8TR	16.93	0.17	28.00	0	0
5C9TR	14.70	0.17	25.83	<1	0
C10TR	4.12	0.17	11.14	4	<1
C11TR	3.54	0.17	13.17	17	7.5
C12TR	1.10	3.00	8.52	20	11
C16TR	0.73	3.00	7.23	46	42

5C9TR, 5-nonanyl treprostinil; AUC, area under the concentration-time curve; C8TR, octyl treprostinil; C10TR, decyl treprostinil; C11TR, undecyl treprostinil; C12TR, dodecyl treprostinil; C16TR, hexadecyl treprostinil; C_{max}, maximum concentration; MDI, metered dose inhaler; PK, pharmacokinetic; T_{max}, time at which the maximum plasma TRE concentration (C_{max}) is observed; TPD, treprostinil prodrug; TRE, treprostinil.

PK parameters after MDI administration of various TPDs at a targeted dose of 100 µg/kg.

Repeat-Dose PK Studies

C10TR, unlike C12TR, was effectively cleared from the lungs 12 h after dosing, with no signs of progressive accumulation (ie, steady plasma TRE and lung TPD levels) over 5 days of bid dosing.



C10TR, decyl treprostinil; C12TR, dodecyl treprostinil; TPD, treprostinil prodrug; TRE, treprostinil.

Figure 3. Plasma TRE (A) and lung TPD equivalents (B) levels after 5 days of bid dosing with either C10TR or C12TR at a targeted dose of 300 µg/kg; TRE and TPD levels were measured 12 h after dosing for 9 dose sessions and every 12 h after the final dose continuing for 72 h. Lung tissues were homogenized for analysis of TPD and TRE. The measure of TPD equivalents represents the molar sum of TPD and TRE in the lung expressed as ng TPD/g tissue.

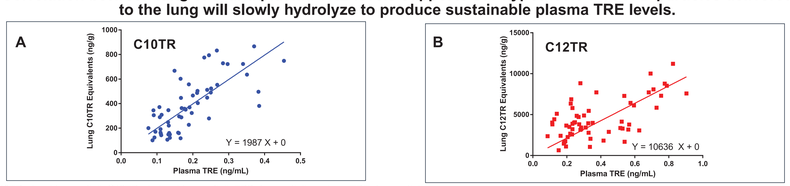
Table 2. Calculated Plasma TRE AUC Values for Doses 1, 6, and 10. Plasma Samples Were Collected Immediately (0.17 h), 1 h, 3 h, 6 h, and 12 h After Dosing

Parameter	C10TR, 300 µg/kg			C12TR, 300 µg/kg		
	Dose 1	Dose 6	Dose 10	Dose 1	Dose 6	Dose 10
Plasma C_{max} (ng/mL)	12.2	12.3	12.7	3.39	5.81	4.97
Plasma T_{max} (h)	0.17	0.17	0.17	0.17	0.17	0.17
Plasma AUC 0-12 (ng*h/mL)	23.9	21.2	28.8	21.3	20.5	21.5

AUC, area under the concentration-time curve; C10TR, decyl treprostinil; C12TR, dodecyl treprostinil; C_{max}, maximum concentration; MDI, metered dose inhaler; PK, pharmacokinetic; T_{max}, time at which the maximum plasma TRE concentration (C_{max}) is observed; TRE, treprostinil.

PK parameters after MDI administration of either C10TR or C12TR at a targeted dose of 300 µg/kg.

The ratio of lung TPD/plasma TRE (slope) indicated faster clearance of C10TR from the lungs than C12TR. Correlation between lung TPD and plasma TRE levels supports our hypothesis that TPD particles delivered to the lung will slowly hydrolyze to produce sustainable plasma TRE levels.



C10TR, decyl treprostinil; C12TR, dodecyl treprostinil; TPD, treprostinil prodrug; TRE, treprostinil.

Figure 4. Correlation between plasma TRE levels and lung TPD levels for C10TR (A) and C12TR (B) after 5 days of bid dosing at targeted doses of 100 µg/kg and 300 µg/kg. All data points correspond to samples collected 12 hours after dosing.

CONCLUSIONS

- By altering the alkyl chain length of TPD drug candidates, we were able to modify the PK profile of TRE derivatives to reduce the peak plasma TRE concentration (C_{max}) and delay its onset (T_{max}) in a predictable, structure-dependent fashion.
- Longer-chain TPDs such as C16TR exhibit delayed kinetics (longer T_{max}, much lower C_{max}, and slow clearance of TPD from lung tissues), whereas TPDs of intermediate chain length (ie, C10TR) exhibit optimized kinetics (slightly longer T_{max}, lower C_{max}, and minimal accumulation in the lungs 24 hours after dosing) relative to TRE when delivered by MDI.
- Based on these data, we believe that further investigation is warranted for treatment of PAH with MDI TPDs.

REFERENCES

1. LeVarge BL. *Ther Clin Risk Manag*. 2015;11:535-547.
2. Data on file. Insmed Incorporated, Bridgewater, NJ.

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DISCLOSURES

All authors are employees of Insmed Incorporated.

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