Effect of TSN3015 on the Development of Pulmonary Arterial Hypertension in Male Sprague-Dawley Rats

Peter B Senese[1], Kimberly R Doherty[1], David Bullough[2], Vinicius Carreira[3], Michael R. Gralinski[1]

[1]CorDynamics, Inc., Chicago, IL; [2]Thesan Pharmaceuticals, Inc., Carlsbad, CA; [3]Vet Path Services, Inc., Mason, OH.

Abstract

TSN3015 (β-naphthoflavone), an AHR agonist with sebosuppressive activity, was under development as an anti-acne medication. In toxicology studies in rats, microscopic changes suggestive of pulmonary arterial hypertension (PAH) were observed in the lungs and hearts of some TSN3015-treated animals. This study was undertaken to determine the effect of TSN3015 on pulmonary artery pressure (PAP). Conscious pulmonary artery-telemeterized male Sprague-Dawley rats were subcutaneously administered either vehicle or TSN3015 at 3 mg/kg for 28 consecutive days, followed by a 55-day recovery period. Clinical observations, telemetry data and blood samples were collected at defined time points throughout the study. The heart and lungs were collected at necropsy for weights and histological examination. No adverse clinical observations were noted over the 85-day study though 2 animals died during the recovery period. A 3 mg/kg dose of TSN3015 elicited an increase in systolic PAP that remained elevated for up to 49 days after the last dose was administered (36.2±8.3 mmHg on Day 84 vs 31.5±2.6 mmHg on Day -1), with the peak increase occurring on Day 49 (range: +34.3% to +117.8%). TSN3015 did not notably affect the diastolic PAP or heart rate. Necropsy findings showed an increase in right heart weight and Fulton Index (FI) in animals given TSN3015, while histological examinations showed microscopic changes in the lungs and hearts of some TSN3015-treated animals. Microscopic findings in the lung consisted of minimal hypertrophy/hyperplasia of the tunica media of medium- and small-size arteries and minimal foamy alveolar macrophages; the heart findings presented as microscopic alterations predominantly in the right ventricle. TSN3015-treated animals with lung pathology had a statistically significant elevation in sPAP and FI when compared to either vehicle-treated animals (p<.001 for both parameters) or TSN3015-treated animals without lung pathology (p<.001 for sPAP, p= .004 for FI). Overall, 9 of the 12 animals treated with TSN3015 developed right heart hypertrophy as indicated by an increased FI (0.373±0.016 TSN-treated animals vs 0.318±0.015 for vehicle-treated animals, p<.05), and these elevated FI values correlated with histologic findings in the heart and lungs. Thus, under the confines of this study, TSN3015 appears to create characteristics consistent with PAH in male rats.

Introduction

Pulmonary arterial hypertension (PAH) is a pathophysiological disorder with no known cure characterized by a narrowing of the pulmonary arteries, leading to increased pulmonary vascular resistance, subsequent right heart hypertrophy and right ventricular failure (Maarman et al 2013, Colvin and Yeager 2014). These clinical signs are observed in animal models as an increase in pulmonary artery pressure (PAP), histological changes to the small pulmonary arterioles, and right ventricular hypertrophy. TSN3015 (β-naphthoflavone), an AHR agonist with sebosuppressive activity, was under development as an anti-acne medication. A toxicology study in rats showed microscopic changes suggestive of PAH in the lungs and hearts of some TSN3015-treated animals. Therefore, this study was undertaken to determine the effect of TSN3015 on PAP and pulmonary vasculature in male rats.

Methods

Experimental Plan

A total of 23 male Sprague-Dawley rats (Charles River) with a mean body weight of 0.371 ± 0.01 kg (range: 0.270–0.448 kg) were used on study. On Study Day 1, rats received a subcutaneous injection (delivered at 1 mL/kg) of either vehicle (corn oil) or TSN3015 at 3 mg/kg. Once-daily dosing continued from Study Day 2 through Study Day 28 (Dosing Phase). Rats were then observed for an additional 55 days (Recovery Phase). Of the 23 rats, 18 were dosed for hemodynamic monitoring (Groups 1 and 2) and 5 were dosed for toxicokinetic sampling (Group 3).

Experimental Design

- A Data Sciences International (St Paul, MN) HD-S21 implantable radiotelemetry device was implanted into each rat by CorDynamics, Inc. scientists to allow for acquisition of defined hemodynamic parameters and body temperature (BT) throughout the study for animals in Group 1 and Group 2.
- Hemodynamic and BT parameters were monitored with the Data Sciences International (Arden Hills, MN) Dataquest A.R.T. Version 4.3 data capture system.
- Data were captured on specified study days from approximately 1.5 hours prior to dosing to 1.5 hours after dosing during the Dosing Phase and for approximately 1.5 hours during the Recovery Phase.
- Hemodynamic parameters were averaged into 5-minute blocks that were then used to create superintervals for analysis.
- Animals were subcutaneously dosed once-daily during the Dosing Phase.
- Dose formulation samples were retained from the first and last day of dosing for analysis.
- Body weights and clinical observations were recorded at protocol-specified time points during both the Dosing Phase and Recovery Phase.
- Blood samples were collected via the retro-orbital venous plexus at protocol-specified time points for animals in Group 3. Plasma was acquired and used for toxicokinetic analysis.
- Macroscopic evaluations were performed on animals found dead prior to the termination of the study.
- All rats were euthanized under ketamine/xylazine anesthesia on Study Day 85.
- The hearts and lungs were collected and processed for histopathological examination.

Toxicokinetic Measurements

- Rat plasma samples were analyzed for TSN3015 by a validated HPLC-MS/MS assay at MicroConstants, Inc (San Diego, CA).
- Descriptive TK parameters were determined by standard model independent methods (Gibaldi and Perrier, 1982) based on the individual plasma concentration-time data for each animal.
- TK analyses of the plasma concentration profiles were performed using noncompartmental analysis with validated Phoenix WinNonlin Professional 6.3 software (Certara, L.P., St. Louis, MO, USA).

Histopathology

- Protocol defined tissues were transferred to Vet Path Services, Inc. (Mason, Ohio) for tissue processing and slide preparation following standard histological techniques and microscopic evaluation.
- Heart and lung tissues from all main study animals at the recovery necropsy (Day 85) and animals found dead were microscopically evaluated.
- Microscopic observations were given a severity score based upon a scale of minimal, mild, moderate and marked. Provantis™ pathology software v9.3.1.1 was utilized for data capture and table generation.
- Macroscopic observations were provided by CorDynamics to VPS for evaluation.

Results

Dose Formulation and Bioanalysis Results

- The overall mean of all dose formulations was within 4% of the target concentration.
- TSN3015 exposure was confirmed in the plasma during the dosing phase, with higher levels reported on Study Day 1 than Study Day 28. TSN3015 levels were below the level of quantification (< 2.00 ng/mL) during the recovery phase.

Morbidity, Mortality and Clinical Observations

- No evidence of morbidity and mortality was noted in the vehicle-treated animals.
- Two TSN3015-treated animals were found dead over the course of the study (n = 1 on Study Day 41; n = 1 on Study Day 69)
- Clinical observations were limited to dermal scabs and abrasions at the dose site.
- No overt changes in body weight were noted for any animal on study.

Toxicokinetic Parameters of TSN3015 Following Repeated Once-Daily Subcutaneous Dosing

TK Parameter	Study Day 1	Study Day 28
C_{max} (ng/mL)	13.5 ± 21.0	0.750 ± 0.380
T_{max} (h)	1.00 ± 0.00	3.80 ± 3.83
$AUC_{(0.5\text{-}8)}$ (ng·h/mL)	24.6 ± 30.6	1.69 ± 1.77

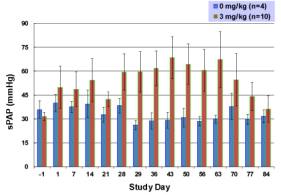
Values are mean ± SD.

On Study Day 1, TSN3015 plasma concentrations reached C_{max} at 1 h after dosing. On Study Day 28, TSN3015 plasma concentrations reached C_{max} at 3.80 h. Both the C_{max} and $AUC_{(0.5\text{-}8)}$ values were lower on Study Day 28 than on Study Day 1.

Hemodynamic Data

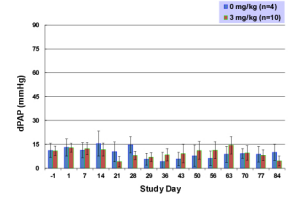
- The study started with 6 animals in the vehicle group and 12 animals in the TSN3015 group; 2 animals were excluded from each treatment group due to aberrant baseline pressure values.

Effect of TSN3015 (subcutaneous injection) on Systolic Pulmonary Artery Pressure in Conscious Rats



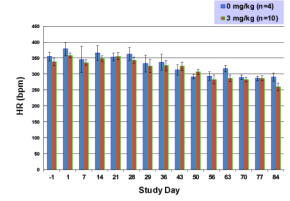
Systolic pulmonary artery pressure (sPAP) was increased by 89% as compared to baseline for TSN3015-treated animals by Study Day 28 (end of dosing phase). sPAP values peaked on Study Day 43 (+118%) in TSN3015-treated animals and remained elevated through Study Day 63 (+114%), after which values began to decline towards BL (+15% on Study Day 84). sPAP values remained relatively stable for vehicle-treated animals throughout the study.

Effect of TSN3015 (subcutaneous injection) on Diastolic Pulmonary Artery Pressure in Conscious Rats



No relevant changes were observed in diastolic PAP values following treatment with either vehicle or TSN3015.

Effect of TSN3015 (subcutaneous injection) on Heart Rate in Conscious Rats



No relevant changes were observed in heart rate values following treatment with either vehicle or TSN3015.

Necropsy Findings, Organs Weights and Histopathology

- TSN3015-treated animals exhibited a mild right heart hypertrophy with a 19% increase in right ventricle weight and a statistically significant 17% increase in Fulton Index when compared to vehicle-treated animals.
- Mild increases in intact heart weight, left ventricle + septum weight, and lung weight were observed in TSN3015-treated animals as compared to vehicle-treated animals.

Comparison of Organ Weight Values Between Vehicle-treated and TSN3015-treated Animals

Treatment	Terminal Body Wt (kg)	Heart Wt (g)	RV Weight (g)	LV+S Wt (g)	Lung Wt (g)	Brain Wt (g)
Vehicle	0.617 ± 0.03	1.5315 ± 0.070	0.3208 ± 0.015	1.1053 ± 0.049	1.8175 ± 0.073	2.1941 ± 0.038
TSN3015 at 3 mg/kg	0.578 ± 0.02	1.5149 ± 0.073	0.3613 ± 0.026	0.9605 ± 0.038	1.8014 ± 0.060	2.1386 ± 0.021

Data presented as mean ± SEM.
LV, left ventricle; RV, right ventricle; S, septum; Wt, weight.

- No TSN3015-related macroscopic observations were noted at recovery necropsy (Study Day 85).
- Microscopic changes were observed in both the heart (predominantly the right ventricle) and lungs of animals treated with TSN3015.
- These microscopic findings correlated with Fulton Index values:
  - A statistically significant elevation in both sPAP and Fulton Index was observed when TSN3015-treated animals with lung pathology were compared to vehicle-treated animals (p = .003)
  - A statistically significant elevation in both sPAP and Fulton Index was observed when TSN3015-treated animals with lung pathology were compared to TSN3015-treated animals without lung pathology (p < .001).

Overall Incidence of Histopathological Findings and Mean Fulton Index Values

	Vehicle[a] (n=6)	TSN3015 3 mg/kg/day (n=10)[b]
PATHOLOGICAL FINDING		
Heart		
Fibrosis, interstitial, myocardium		
minimal	FNP	2
Increased cellularity, endocardium		
minimal	FNP	3
Increased cellularity, atrioventricular valve		
minimal	FNP	3
mild	FNP	1
Hypertrophy/hyperplasia, tunica media, artery;		
minimal	FNP	1
Hypertrophy, myocardium		
minimal	FNP	1
mild	FNP	2
Lungs		
Hypertrophy/hyperplasia, tunica media, artery		
minimal	FNP	4
Foamy alveolar macrophages		
minimal	FNP	4
Inflammation, subacute; perivascular		
minimal	FNP	1
Pigmented macrophages; alveoli		
minimal	FNP	1
FULTON INDEX (Mean ± SEM)		
	0.3176 ± 0.0145	0.3730 ± 0.0155

[a]Vehicle was 100% corn oil.
[b]Two animals (#259 and #261) were not included in these analyses due to early death.

Conclusions

Overall, 3 mg/kg TSN3015 injected subcutaneously once daily elicited an increase in sPAP without eliciting effects on either dPAP or HR. The lack of effect on HR indicated that minimal or no changes to systemic blood pressures occurred following administration of TSN3015. These hemodynamic data suggest that the effects of TSN3015 were likely due to pulmonary vascular constriction. Necropsy findings showed an increase in right ventricle weight and an increase in the Fulton Index in TSN3015-treated animals. Further, higher Fulton Index values correlated with the microscopic histopathological findings in the heart and lungs of TSN3015-treated animals as compared to vehicle-treated animals. Indeed, the relationship between the observed lung pathology and either sPAP or Fulton Index in TSN3015-treated animals compared to vehicle-treated animals was statistically significant. Thus, under the confines of this study, 3 mg/kg/day administration of TSN3015 induced an elevated sPAP, right heart hypertrophy and histological changes consistent with pulmonary hypertension in rats.

References

Colvin KL and Yeager ME. Animal models of pulmonary hypertension: matching disease mechanisms to etiology of the human disease. *J Pulm Respir Med.* 2014Aug 4;4(4). pii: 198.

Maarman G, Lecour S, Butrous G, Thienemann F, Sliwa K. A comprehensive review: the evolution of animal models in pulmonary hypertension research; are we there yet? *Pulm Circ.* 2013; 3(4): 739-756.

Gibaldi, M. and Perrier, D. Pharmacokinetics, Second Edition, Marcel Dekker, Inc., New York.

www.cordynamics.com