

RESEARCH LETTER

TPT-004, a Next-Generation Inhibitor of Tryptophan Hydroxylase, Ameliorates Pulmonary Arterial Hypertension in Rats

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Pulmonary arterial hypertension (PAH) is a multifaceted cardiovascular syndrome characterized by pronounced remodeling of the pulmonary vasculature and progressive elevation in pulmonary vascular load, resulting in the restructuring of the right ventricle. Untreated progression of PAH ultimately leads to fatal right ventricular (RV) failure. Despite its severity, therapeutic options are limited and fail in their primarily symptomatic approach to target the underlying cause, often leaving lung transplantation as the sole lifesaving option. Novel interventions addressing the underlying pathological mechanisms of PAH are urgently needed to treat its devastating outcomes.

Animal studies and observations in human patients have shown that peripheral serotonin plays a significant role in the development and progression of PAH.¹ Elevated serotonin in pulmonary arteries contributes to vasoconstriction, proliferation of smooth muscle cells, and inflammation, collectively leading to pulmonary vascular remodeling, a hallmark of PAH. Mice lacking the serotonin-producing enzyme tryptophan hydroxylase 1 (TPH1) show protection against PAH.¹ Chemical inhibition of TPH1, reducing peripheral serotonin, presents an innovative therapeutic approach for PAH and related diseases.² Notably, TPH inhibitors have demonstrated efficacy in rodent PAH models and are currently undergoing clinical trials.³

We have developed a novel class of xanthine–imidazopyridine and –imidazothiazole TPH inhibitors to reduce pathologically high serotonin synthesis in the periphery. They use an active drug approach and enhanced double binding mode to target both catalytic pockets (tryptophan and tetrahydrobiopterin) of TPH1, distinguishing them from existing TPH inhibitors that are currently on the market (telotristat ethyl) or under clinical investigation (rodatristat ethyl).^{4,5} In this study, we evaluate the efficacy of our lead compound, TPT-004, as an oral monotherapy for PAH.

The data that support the findings of this study are available from the corresponding authors upon reasonable request. TPT-004 was assessed in the Sugen-Hypoxia (SuHx) rat model, adequate for studying severe PAH and RV failure. The study enrolled 37 male Sprague Dawley rats (Charles River Laboratories, USA), 8–9 weeks old. Inclusion criteria (sex, appropriate body weight, absence of behavioral changes) were established prior to the study commencement. The study director was cognizant of group allocation throughout the experiment. The blinding procedure extended to veterinary and laboratory technicians responsible for treatment administration and terminal surgery. Control animals in the normoxia group (n=5) received a subcutaneous injection of 100% DMSO (2 mL/kg) on Day 0 and were exposed to ambient oxygen for 56 days.

Key Words: pulmonary arterial hypertension ■ serotonin ■ TPH inhibitor ■ tryptophan hydroxylase

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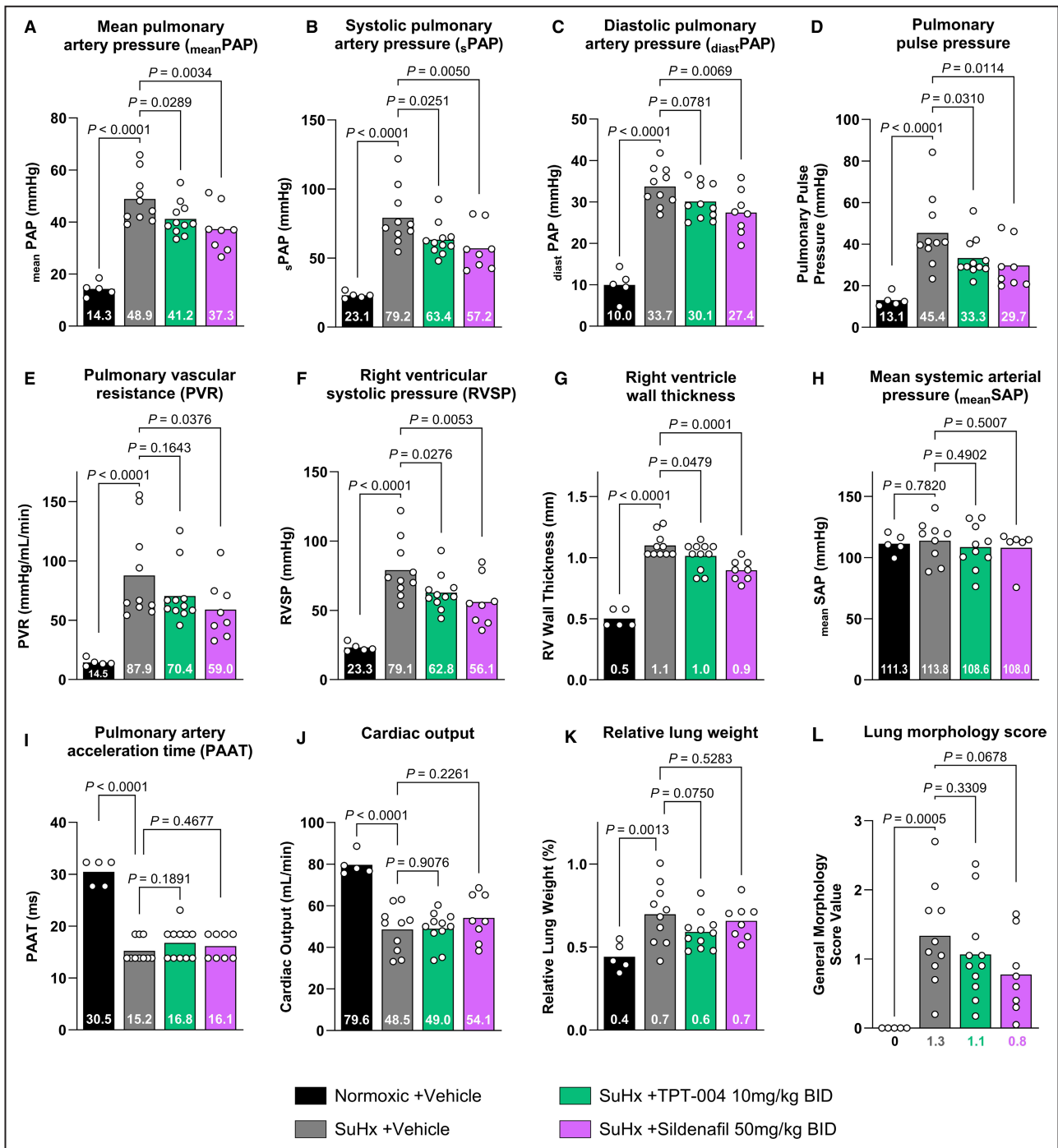


Figure. Therapeutic efficacy of TPT-004 in Sugen-Hypoxia rat model of PAH.

Hemodynamic parameters after 5-week TPT-004 treatment in Sugen-Hypoxia rat model of PAH. (A) Mean PAP; (B) systolic PAP; (C) diastolic PAP; (D) pulmonary pulse pressure; (E) PVR; (F) RVSP; (G) right ventricular wall thickness; (H) mean SAP; (I) PAAT; (J) cardiac output; (K) relative lung weight; (L) lung morphology score. SAP was recorded via an intra-arterial fluid-filled catheter inserted in the femoral artery; RVSP and PAPs were recorded via an intraventricular fluid-filled catheter (both AD Instruments, Colorado Springs, CO) using LabChart 8. An echocardiograph (Model Vivid 7, GE Healthcare, Chicago, IL) connected to an i13L 10.0MHz probe was used to measure heart rate and PAAT. In hematoxylin and eosin-stained lung sections, the general morphology of alveolar septa, lung structure, and inflammation was scored in a blinded manner according to Bao et al.⁶ Mean values are depicted at the base of the bars. One-way ANOVA test with Fisher least significant difference post hoc multiple comparisons test determined by Prism 10 (GraphPad Software, La Jolla, CA). PAAT indicates pulmonary artery acceleration time; PAH, pulmonary arterial hypertension; PAP, pulmonary artery pressure; PVR, pulmonary vascular resistance; RVSP, right ventricular systolic pressure; and SAP, systemic arterial pressure.

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SuHx rats, injected with the vascular endothelial growth factor receptor antagonist, Sugen 5416 (20 mg/kg), on Day 0, were subjected to 3 weeks of hypoxic gas exposure (fraction of inspired oxygen, 0.10) before randomization based on weight and echocardiography results. No surviving animals were excluded from the study after the randomization. After hypoxia, SuHx rats were treated with either vehicle (0.5% CMC-Na) (n=12), sildenafil (50 mg/kg BID = 100 mg/kg/day) (n=8), or TPT-004 (10 mg/kg BID = 20 mg/kg/day) (n=12) for 5 weeks. Terminal surgery, including echocardiogram monitoring and cardiac catheterization, occurred on Day 56, followed by animal euthanasia. Animal work adhered to Association for Assessment and Accreditation of Laboratory Animal Care International guidelines at IPS Therapeutique Inc.'s (Sherbrooke, Quebec, Canada) accredited laboratory facility.

Five-week oral treatment with TPT-004 (20 mg/kg per day) resulted in an improvement of several key hemodynamic parameters, including a reduction of pathologically high mean pulmonary artery pressure by 16% (41.2 mmHg versus 48.9 mmHg in the vehicle group; $P=0.0289$) (Figure [A]). Systolic and diastolic pulmonary artery pressure values were lower with TPT-004 treatment compared with the SuHx+vehicle group (63.4 versus 79.2 mmHg; $P=0.0251$ and 30.1 versus 33.7 mmHg, $P=0.0781$, respectively) (Figure [B] and [C]). Notably, we observed a strong reduction of pulmonary pulse pressure by $\approx 26\%$ (33.3 mmHg versus 45.4 mmHg in the vehicle group; $P=0.031$) (Figure [D]), as well as $\approx 20\%$ lower pulmonary vascular resistance after TPT-004 treatment (70.4 mmHg/mL per min versus 87.9 mmHg/mL per min in the vehicle group; $P=0.1643$) (Figure [E]). Moreover, beneficial effects of TPT-004 were also demonstrated by lowering RV systolic pressure by $\approx 20\%$ (62.8 mmHg versus 79.1 mmHg in the vehicle group; $P=0.0276$) (Figure [F]) and decreasing RV wall thickness (7% reduction; $P=0.0479$) (Figure [G]). Mean systemic arterial pressure (Figure [H]) was similar in all groups; pulmonary artery acceleration time (Figure [I]) and cardiac output (Figure [J]) were equally reduced in all SuHx groups.

The survival rate was 91.7% (11/12) in the TPT-004 group compared with the vehicle group's 83.3% (10/12) over the 56-day in-life phase. Relative lung weight trended toward improvement compared with the SuHx+vehicle group ($P=0.075$), suggesting reduced lung edema (Figure [K]). Additionally, lung morphology scores tended to be improved in the TPT-004 group (Figure [L]).

Our evaluation of TPT-004 in the SuHx PAH model revealed its potential as a novel and promising therapeutic agent for alleviating PAH. The monotherapy with TPT-004 at 20 mg/kg per day significantly enhanced

multiple crucial hemodynamic parameters, indicating an improvement in cardiac dysfunction associated with RV hypertrophy induced by the SuHx challenge. Overall, the impact of TPT-004 at this dosage closely resembled the therapeutic effect achieved by sildenafil at 100 mg/kg per day. Future investigations involving TPT-004 should explore further dose optimization, potential sex-specific efficacy differences, combination treatment with standard-of-care vasodilators, and novel administration routes, including inhalation therapy.

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