Sildenafil versus Ambrisentan in a Rat Model of Pulmonary Arterial Hypertension

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Abstract

We have previously demonstrated the development of increased pulmonary artery pressure (PAP) and hypertrophy of pulmonary vascular smooth muscle akin to pulmonary arterial hypertension (PAH) after a single dose of semaxanib and hypoxia. Further, partial reversal of PAH symptoms has been shown following treatment with sildenafil. This study was undertaken to compare the effectiveness of sildenafil versus the selective type-A endothelin receptor antagonist ambrisentan in this model. Male Sprague-Dawley rats (223−300 g) received a single dose of semaxanib (200 mg/kg SC) on Day 1 and then were placed in a low oxygen (13%) chamber from Day 1−21. Rats were housed in normoxic conditions from Day 21−56. Test compounds were orally dosed from Day 28−56, with sildenafil given at 60 mg/kg/day (BID) and ambrisentan given at 10 mg/kg/day (SID). PAP, arterial pressure and thoracic organ weights were measured on Day 56. Relative to vehicle, PAP decreased more with ambrisentan than with sildenafil. Systolic PAP decreased 11% following sildenafil and 29% following ambrisentan (diastolic PAP: -33% and -33%, mean PAP: -24% and -31%, respectively). Arterial blood pressures and heart rate were similar to vehicle-treated animals. Necropsy findings showed a decrease in right ventricle (RV) weight and Fulton's Index in animals given either sildenafil or ambrisentan. RV weight decreased by 22% and 30% while Fulton's Index decreased by 17% and 25%, respectively. Under the conditions of this study, ambrisentan appears to be a reliable positive control in this model of PAH.

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. Sildenafil, a phosphodiesterase-5 (PDE5) inhibitor, is approved for the treatment of PAH (Parikh et al 2019) and is commonly used as a positive control in assessing the effectiveness of test compounds in PAH studies. Ambrisentan, a selective type-A endothelin receptor antagonist, is also an approved treatment for PAH (Parikh et al 2019) but is less common in preclinical studies. Therefore, this study was undertaken to compare the effectiveness of sildenafil versus ambrisentan in male Sprague-Dawley rats.

Methods

Experimental Plan

A total of 45 male Sprague-Dawley rats (Charles River) with a mean body weight of 0.278 ± 0.003 kg (range: 0.223−0.300 kg) were used on study. On Study Day 1, rats received a subcutaneous injection of SU5416 (semaxanib, 200 mg/kg) and were then housed for 21 days in a low oxygen chamber (~13% O_2) to induce PAH. Rats were removed from the low oxygen chamber on Study Day 21 and then were housed in normoxic conditions until the Day 56 terminal procedure.

A total of 15 animals were assigned to 3 dosing groups with dosing beginning on Study Day 28. Animals in the vehicle group were dosed once-daily with 10% hydroxypropyl-β-cyclodextrin in deionized water. Animals in the sildenafil group were dosed twice daily at 60 mg/kg/day, with approximately 6 hours between dosing times. Animals in the ambrisentan group were dosed once daily at 10 mg/kg. Dosing continued from Study Day 28 through Study Day 56.

Experimental Design

- On Study Day 56, rats were anesthetized via an IP injection of ketamine/xylazine and placed on a heating pad to maintain body temperature. Once consciousness was lost, both a Millar catheter and a fluid-filled catheter were placed to measure arterial blood pressure and pulmonary arterial pressure, respectively.
- Hemodynamic measurements were continuously monitored with the Notocord HEM (Croissy sur Seine, France) v3.5 data capture system.
  - Post-equilibration average values were taken from a 10−15 second block of consecutive cardiac cycles uninterrupted by interference of ectopic beats during the fifteenth minute of the monitoring period for analysis.
  - Values from individual animals were pooled for analysis.
- Body weights and clinical observations were recorded at protocol-specified time points from Study Day 1 to Study Day 56.
- All rats were euthanized under ketamine/xylazine anesthesia on Study Day 56, and the hearts and lungs were removed and gently infused with saline via the vasculature.
  - Heart were weighed, with the following weights recorded: intact heart, right ventricle, left ventricle + septum (LV+S).
  - Lungs were weighed and processed for histopathological examination.

Histopathology

- Protocol-defined tissues were transferred to Seventh Wave Laboratories (Maryland Heights, MO) for tissue processing and slide preparation following standard histological techniques and microscopic evaluation.
- Lung tissues from 40 animals (vehicle: n = 11; sildenafil: n = 14; ambrisentan: n = 15) were processed and stained with α-smooth muscle actin (SMA)/elastin as well as H&E for assessment.
- Arterioles were categorized as completely muscularized, partially muscularized or non-muscularized based on the αSMA/elastin staining.
- The H&E-stained lung sections were examined microscopically and histopathological findings of alveolar infiltrate, perivascular/vascular inflammation/fibrosis, vascular necrosis/thrombosis and smooth muscle hypertrophy of small arterioles were scored on a 0–4 scale.

Results

Morbidity, Mortality and Clinical Observations

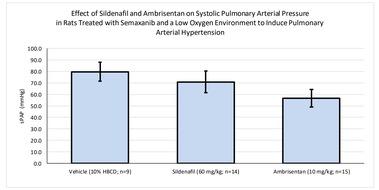
- Four vehicle-treated animals were found dead prior to the terminal procedure (n = 1 on Study Day 47; n = 1 on Study Day 50; n = 2 on Study Day 52).
- One sildenafil-treated animal was found dead prior to the terminal procedure (n = 1 on Study Day 42).
- No evidence of morbidity or mortality was noted in the ambrisentan-treated animals.
- Clinical observations were limited to dermal scabs and abrasions at the dose site, alopecia and staining around the nares.
- One primary necropsy observation of fluid in the thoracic cavity, ranging from 1−6 mL, was noted in all 3 study groups.
  - Group 1, n = 5/15; Group 2, n = 3/15; Group 3, n = 2/15
- Body weight gain improved by 18% over the study duration in animals treated with either sildenafil or ambrisentan when compared to vehicle-treated animals.

Hemodynamic Data

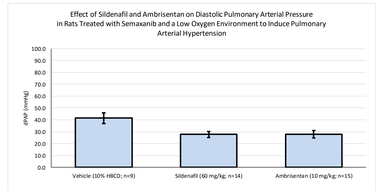
- The study started with 15 animals in each study group.
  - A total of 11 animals completed the study in the vehicle group, 14 animals completed the study in the sildenafil group and 15 animals completed the study in the ambrisentan group. Hemodynamic data from only 9 animals in the vehicle group were analyzed due to deaths during the terminal procedure.
- Systolic pulmonary artery pressure (sPAP) was decreased by 11% following sildenafil treatment and by 29% following ambrisentan treatment when compared to the vehicle.
- Diastolic PAP and mean PAP were also decreased compared to vehicle
  - Sildenafil: -33% and -24%, respectively
  - Ambrisentan: -33% and -31%, respectively
- Arterial blood pressures (MAP, SAP and DAP) and heart rate were similar among all groups.

Effect of Sildenafil and Ambrisentan on Pulmonary Arterial Pressures in Sprague-Dawley Rats

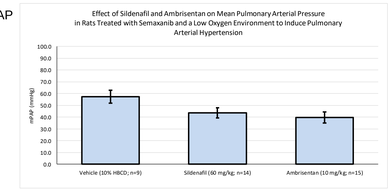
A. sPAP



B. dPAP

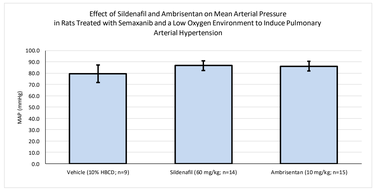


C. mPAP

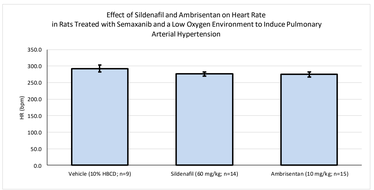


Systolic, diastolic and mean pulmonary arterial pressures decreased following exposure to either sildenafil or ambrisentan when compared to vehicle.

Effect of Sildenafil and Ambrisentan on Mean Arterial Pressure in Sprague-Dawley Rats



Effect of Sildenafil and Ambrisentan on Heart Rate in Sprague-Dawley Rats



Necropsy Findings, Organs Weights and Histopathology

- Sildenafil elicited a 14% decrease in intact heart weight, a 22% decrease in right ventricle weight and a 17% decrease in the Fulton Index when compared to vehicle.
- Ambrisentan elicited an 18% decrease in intact heart weight, a 30% decrease in right ventricle weight and a 25% decrease in the Fulton Index when compared to vehicle.
- Mild changes were noted in the LV+S weight and lung weight for both test articles when compared to vehicle.

Comparison of Organ Weight Values Between Vehicle-treated, Sildenafil-treated and Ambrisentan-treated Animals

Treatment	Terminal Body Wt (kg)	Heart Wt (g)	RV Wt (g)	LV+S Wt (g)	Lung Wt (g)
Vehicle	0.488 ± 0.019	2.0802 ± 0.0945	0.6515 ± 0.0450	1.1161 ± 0.0374	2.3013 ± 0.0822
Sildenafil	0.476 ± 0.015	1.7809 ± 0.0880	0.5058 ± 0.0431	1.0339 ± 0.0311	2.2950 ± 0.0687
Ambrisentan	0.476 ± 0.013	1.7135 ± 0.0909	0.4552 ± 0.0476	1.0292 ± 0.0331	2.2231 ± 0.0902

LV, left ventricle; RV, right ventricle; S, septum; Wt, weight.

- Substantial decreases in muscularization were observed in the lungs of ambrisentan-treated animals but not in the lungs of sildenafil-treated animals when compared to vehicle-treated animals.
- Both sildenafil and ambrisentan treatment resulted in a decrease in the percentage of fully obstructed arterioles when compared to vehicle-treated animals.

Muscularization and Obstruction of Pulmonary Arterioles

Treatment	Pulmonary Arterioles (%)					
	Non-Muscularized	Partially Muscularized	Completely Muscularized	Not Obstructed	Partially Obstructed	Fully Obstructed
Vehicle	3.5	24.2	72.4	38.0	45.2	16.8
Sildenafil	3.4	29.4	67.1	47.7	42.9	9.4
Ambrisentan	6.7	38.3	54.9	57.8	36.0	6.2

Histopathological Staining of Lung Tissue: Vehicle vs Ambrisentan

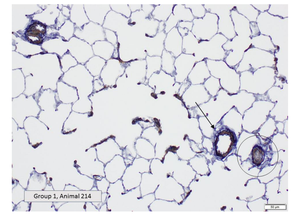


Figure 1. Vehicle-treated animal. Image of α-smooth muscle actin (20× objective). A fully muscularized (upper left), partially vascularized (arrow) and a completely obstructed, fully muscularized arteriole (circled) are present.

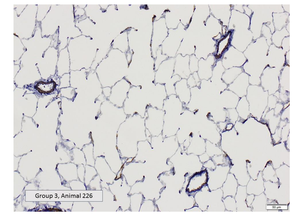


Figure 2. Ambrisentan-treated animal. Image of α-smooth muscle actin (20× objective). Compared to vehicle controls, obstruction was often not present in fully muscularized (left) and partially muscularized (right) arterioles following administration of ambrisentan.

Conclusions

In conclusion, 5 rats were found dead (Vehicle: n = 4; Sildenafil: n = 1) prior to the scheduled terminal procedure on Study Day 56. Clinical observations were minor consisting predominantly of soft or firm masses or scabs/lesions found at subcutaneous injections sites and porphyrin staining around nares. One main necropsy finding of fluid in the thoracic cavity was noted in at least one animal per group. Treatment with ambrisentan at 10 mg/kg/day and sildenafil at 60 mg/kg/day attenuated the elevated pulmonary artery pressure (as reflected in sPAP) and reduced right heart hypertrophy (as reflected in the Fulton Index) while minimally impacting arterial blood pressures. Decreases in muscularization and partially/fully obstructed arterioles occurred following the administration of ambrisentan and were more substantial compared to the administration of sildenafil. Therefore, ambrisentan appears to be a reliable positive control in this model of PAH.

References

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