The Cardiovascular Effects of Tobacco Smoke Exposure in a Rat Model of Chronic Obstructive Pulmonary Disease

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Introduction

Cigarette smoking is the leading cause of preventable disease and death in the United States, accounting for more than 480,000 deaths every year, or 1 of every 5 deaths.[4] Smoking causes a number of diseases, including cancers, myocardial infarction, stroke, and chronic obstructive pulmonary disease (COPD). COPD is becoming increasingly prevalent globally, and is expected to become the third leading cause of death worldwide by 2020.[1]

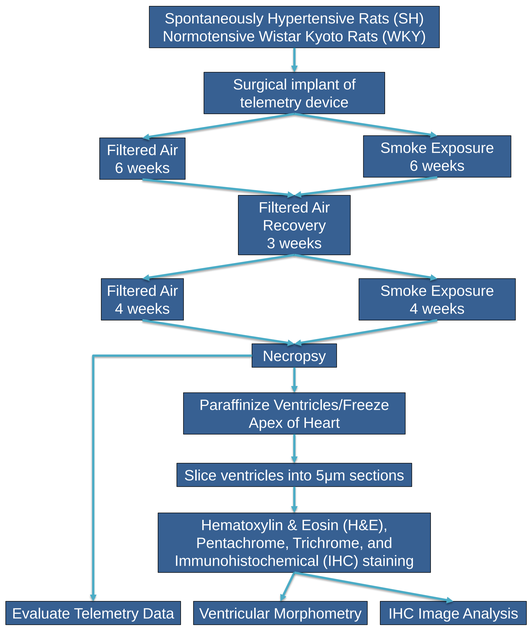
Pulmonary and systemic hypertension are significant causes of morbidity and mortality in patients with COPD. Factors suspected to influence pulmonary hemodynamics in COPD patients include lung parenchyma destruction with associated loss of pulmonary microvasculature, and pulmonary artery vasoconstriction induced by alveolar hypoxia. Pulmonary hypertension in COPD is believed to promote structural vascular changes, which is supported by the failure of oxygen therapy to reverse it.[2] However, very little is understood regarding how progressive physiological changes of the cardiovascular system may affect resultant changes in cardiovascular morphology.

Cardiovascular changes are commonly associated with COPD. Certain polymorphisms found in COPD patients are implicated in increasing risks for other types of diseases, including cancer and cardiovascular disease.[3] Cardiovascular symptoms may appear in the form of atherosclerosis, myocardial infarcts and/or systemic hypertension. Chronic exposure of the spontaneously hypertensive (SH) rat to tobacco smoke results in COPD-like lung inflammation, airway obstruction, mucous hypersecretion and emphysematous changes. Little is known regarding the cardiovascular impacts in this animal model, although telemetric measures of cardiovascular physiology are available, along with heart tissue samples. Therefore, this study addresses this critical deficit by analyzing telemetric data in correlation to structural cardiovascular change.

Hypotheses

1. Exposure to tobacco smoke alters cardiac function and morphology in spontaneously hypertensive rats.
2. Spontaneously hypertensive and normotensive Wistar Kyoto rats show differential sensitivity to tobacco smoke.

Methods



Results

Telemetry Data

Figure 1. Telemetric Linear Analysis

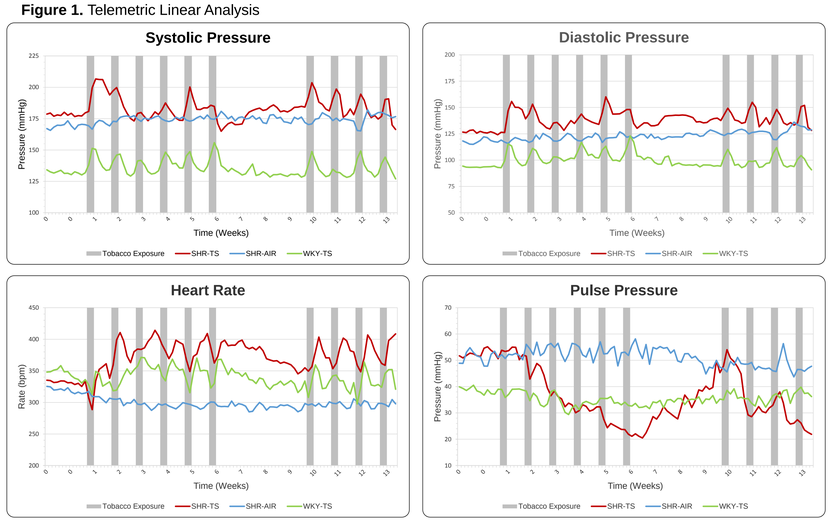


Table 1. Statistical analysis of telemetry data

ANOVA Mixed Effects Analysis of Variance

The effect of exposure in SH rats

Physiologic Parameter	Exposure	M	95% CI
Heart Rate (bpm)	N	371.55**	364.89-378.21
	Y	369.67	362.98-376.36
Systolic Pressure (mmHg)	N	179.71	167.80-191.63
	Y	189.63**	177.72-201.55
Diastolic Pressure (mmHg)	N	135.20	124.87-145.52
	Y	145.31**	134.98-155.64
Pulse Pressure (mmHg)	N	44.43**	40.81-48.05
	Y	44.17	40.55-47.80

n=59,678

The effect of strain in tobacco exposed rats

Physiologic Parameter	Strain	M	95% CI
Heart Rate (bpm)	SHR	369.61**	362.70-376.53
	WKY	341.35	332.43-350.28

n=23,395

*Note: **= p<0.001, M= mean predicted value, N= not under the influence of tobacco smoke, Y= under the influence of tobacco smoke. Heart rate was limited to values ≥ 200bpm*

Figure 1. Graphs representing physiological parameters measured through telemetric devices. Daily averages were calculated for each group of rats (SHR-TS= 5, SHR-AIR= 4, WKY-TS= 3) and plotted on graphs. These graphs highlight transient physiological changes produced by tobacco exposure, as well as the long-term effects of tobacco exposure between exposure groups and strains.

Table 1. Statistical results of the telemetric data using ANOVA and a mixed-effects analysis of variance. This table emphasizes the effects within the SH rats that were intermittently exposed to tobacco smoke, as well as the effect of tobacco exposure on heart rate between strains.

Troponin I Immunohistochemistry and IHC Profiler

Figure 2. Immunohistochemical staining analysis by ImageJ

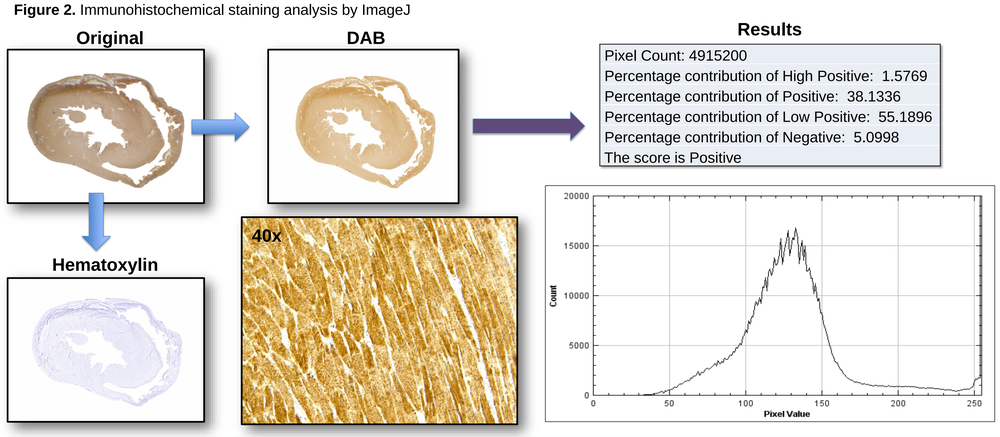


Figure 2. Sample IHC stained slide from an SH rat exposed to tobacco smoke, as well as a 40x magnified section of the right ventricular wall. 5µm sections were taken from SH and WKY rats exposed to tobacco smoke, and SH rats exposed to filtered air. Slides were stained with Cardiac Troponin I (cTnI) antibody using standard IHC protocols. Images were analyzed in the ImageJ IHC Profiler. Four intensity ranges were used for scoring the detection of DAB: high-positive (0-60), positive (61-120), low-positive (121-180), and negative (181-255). Sample numeric and histogram results are shown. Two-tailed Student's T-tests were used to quantitatively determine significance. There was no significant difference between strains or exposure groups.

Morphologic and Morphometric Data

Figure 3. H&E staining of ventricles

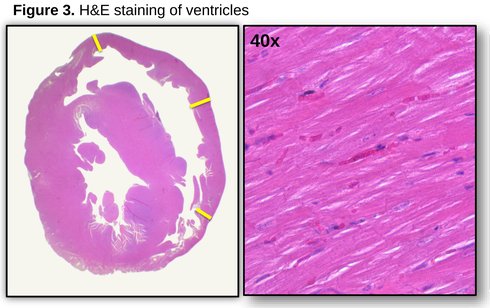
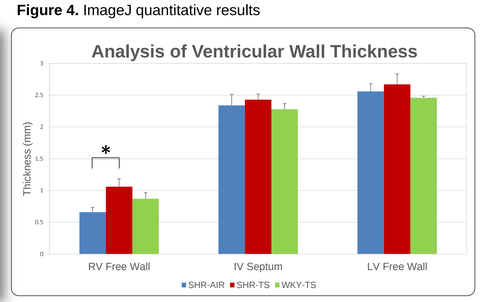


Figure 3. Sample images of an H&E stained slide. The first image is the entire cross-sectional area of the ventricles from an SH rat exposed to tobacco smoke. The lines show where three ImageJ measurements were taken to determine ventricular wall thickness. An average wall thickness was calculated using three slides from each rat, or nine total measurements. The second image shows a magnified image at 40x from the same rat.

Figure 4. Quantitative results calculated in ImageJ using mean values. RV= right ventricle, IS= interventricular septum, LV= left ventricle. *= p<0.02.

Figure 4. ImageJ quantitative results



Histological Stains

Figure 5. Sample Trichrome and Pentachrome stained slides

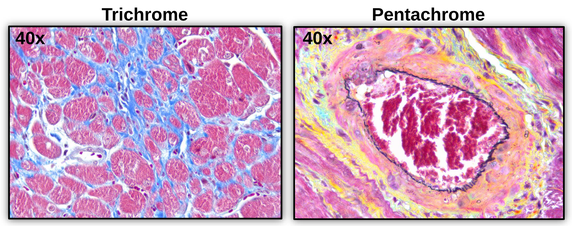


Figure 5. Samples of Trichrome and Pentachrome stained slides captured at 40x from SH rats exposed to tobacco smoke intermittently for up to 16 weeks. Trichrome stained images were used to analyze the ventricular myocardium. Pentachrome stained images were used to analyze the coronary microvasculature. There were no qualitative differences between the tobacco-exposed and unexposed groups.

Conclusions

- In both SH and WKY rats, exposure to tobacco smoke was associated with significant transient increases in systolic and diastolic blood pressure.
- In WKY rats, all physiological parameters returned to baseline levels during non-exposure periods.
- In SH rats, all physiological parameters returned to baseline levels during non-exposure periods, except heart rate, which persisted at an elevated level.
- In SH rats, mean pulse pressure was reduced as a result of a greater increase in diastolic compared to systolic pressure. A progressive decrease in mean pulse pressure in SH rats exposed to tobacco smoke was suggestive of potential heart failure.
- In SH rats, exposure to tobacco smoke caused a statistically significant increase in right ventricular wall thickness compared to the unexposed rats.
- Tobacco exposure produced significantly increased heart rate values in SH and WKY rats. However, the SH rats' heart rates did not return to baseline during the cessation period, which suggests that they are more sensitive to tobacco smoke than WKY rats.
- Telemetric approaches can be implemented to detect and document novel and progressive cardiovascular impacts of tobacco smoke under experimental conditions.

References

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