Establishment of a novel model of group 3 pulmonary hypertension induced by SU5416/hypoxia in rats

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COI Disclosure: The author have no financial conflicts of interest to disclose concerning the presentation.

Background

- Pulmonary hypertension (PH) due to chronic lung diseases (PH-lung, WHO group 3) is associated with increased morbidity and mortality. Previous studies demonstrated that chronic treatment of rats with vascular endothelial growth factor (VEGF) receptor blocker, SU5416, led to enlargement of air spaces of lungs in the rats, indicative of formation of emphysema [J Clin Invest 2000;11:1311-1319].

However, other studies revealed that the SU5416/hypoxia/normoxia-exposed rats developed severe PH of group 1 [Circulation 2010;121(25):2747-2754].

- The aim of this study is to establish SU5416/hypoxia-induced PH in rats which was characterized by chronic pulmonary hemodynamic deterioration in association with emphysema.

Materials and Methods

Animals

Male Sprague-Dawley rats (n=12, 4wk ; Charles River laboratories, Yokohama, Japan.Inc.) were randomly divided into three groups: control , SU5416+Hypoxia group (SuHx), Hypoxia group (Hx). Rats were injected subcutaneously with SU5416(20mg/kg) on day 1, 8 and 15, and then exposed to normobaric hypoxia(15%) for 6 weeks.
Animals were sacrificed on 6weeks after the first SU5416 injection. All the experimental and control rats survived during the experimental period.

Hemodynamic Measurements in Catheterized Rats

Rats were anesthetized with pentobarbital sodium , intubated, and placed in a supine position and ventilated. Hemodynamic measurements were performed under normoxic conditions. After a median sternotomy, right ventricle (RV) outflow tract was punctured with a 23-G needle. Polyvinyl catheters were inserted into the RV and pulmonary artery through the incision. The RV pressure and pulmonary arterial pressures (mPAP) were continuously monitored and recorded by PowerLab®. At the end of each hemodynamic study, the rat was euthanized by an overdose of pentobarbital sodium, and lungs and hearts were collected for histological and immunohistochemical evaluation and RV/LV+septum (RV/LV+S) weight ratio measurement.

Histopathology and Immunohistochemistry

The lungs were inflated with 0.5% agarose at 25cm H_2O pressure and fixed in 10% formalin for 48 hours. The left lobe was blocked and embedded in paraffin, and stained with hematoxylin and eosin (HE) or elastic fiber specific staining . Antibodies used were α-smooth muscle actin (αSMA, Abcam), cleaved Caspase-3 (Cell Signaling Technology), . Emphysema was quantified by measuring the mean linear intercept (MLI) and determining the destructive index (DI).
After immunohistochemical stain of active caspase-3, the Apoptotic Index(AI) was calculated as the percentage of caspase3-positive nuclei. 10 random lung fields per tissue section were captured at x400 magnification. Then the number of active caspase 3-positive cells were counted.

Western Blot Analyses of lung tissue for caspase3 and eNOS

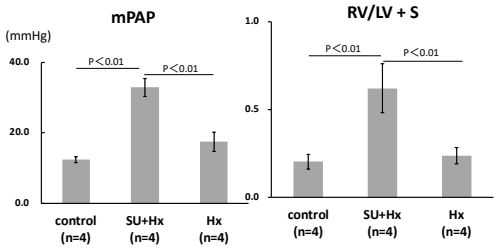
Pulmonary cytoplasmic protein expression of eNOS and β-actin was analyzed by Western blot analyses. The nuclear protein expression of cleaved Caspase-3 and LaminB was also analyzed .

Real-time RT-PCR analysis of lung tissue

Total RNA was extracted and purified from lung tissue using NucleoSpin® RNA Plus(Qiagen). Quantitative real-time PCR was performed on a StepOne Real Time PCR System with TaqManR Gene Expression Assays on Demand probes. 18srRNA was used for internal control.

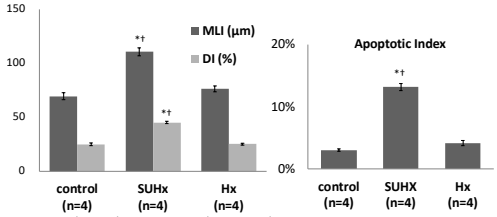
Results

Figure 1. Hemodynamic Data and RV Hypertrophy



mPAP, pulmonary arterial pressures; RV/LV+S, right ventricle (RV)-to-left ventricle (LV) plus septum (S) heart weight ratio; SUHx, SU5416+Hypoxia group; Hx, Hypoxia group. (one-way analysis of variance followed by Tukey-Kramer multiple comparison test.) Results are expressed as mean±SEM (n=4 each).

Figure 2. MLI/DI and Apoptotic index



MLI, measuring the mean linear intercept; DI, destructive index; SUHx, SU5416+Hypoxia group; Hx, Hypoxia group. *P < 0.01 compared with control group; †P < 0.01 compared with Hypoxia group. (one-way analysis of variance followed by Tukey-Kramer multiple comparison test.) Results are expressed as mean±SEM.

Figure 3. Histopathology & Immunohistochemistry

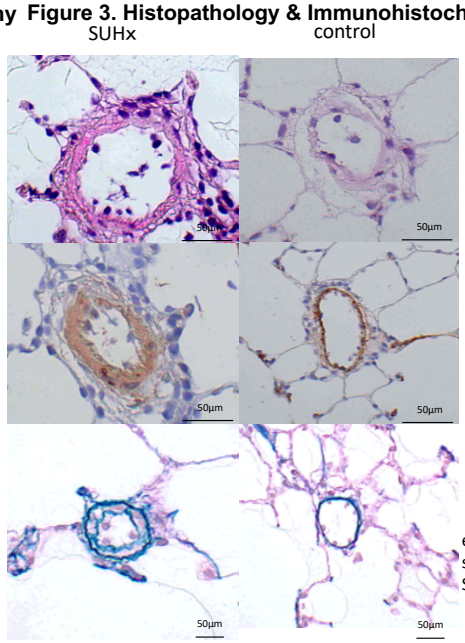
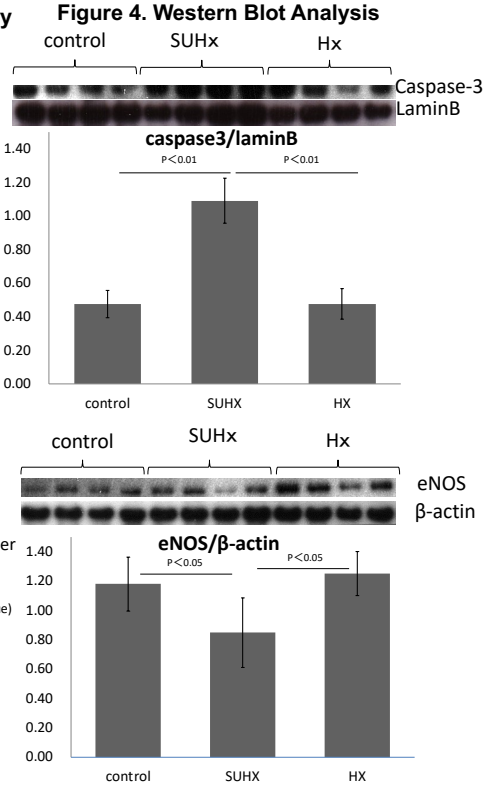
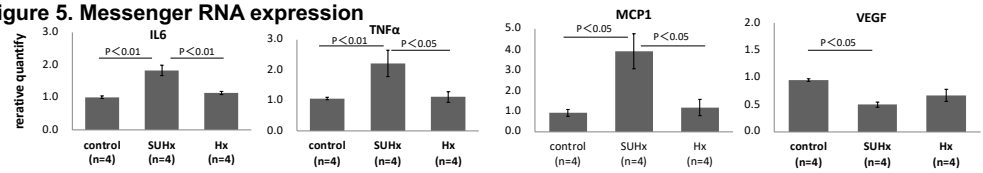


Figure 4. Western Blot Analysis



Caspase level is shown as percent of the internal control LaminB. eNOS level is shown as percent of the internal control β-actin level. Results are expressed as mean±SEM (n=4 each).

Figure 5. Messenger RNA expression



Messenger RNA expression level of IL6, TNF-α, MCP1, VEGF was compared among Sugen/hypoxia (SUHx), hypoxia (Hx), and control rats at 6 weeks after initial treatment. Data was analyzed by Comparative CT (ΔΔCT) . Values are rerative quantify (RQ)compared with the control . mean ± SEM (n=4 each).

Summary of Results

Hemodynamic Data and RV Hypertrophy

The SUHx rats showed PH which was determined by significantly increased mean pulmonary arterial pressure (33 ± 12 mmHg vs. 16 ± 8 mmHg, P < 0.01) and right ventricle (RV)-to-left ventricle (LV) plus septum (S) heart weight ratio (RV/LV + S) (0.71 ± 0.05 vs. 0.20 ± 0.06, P < 0.01) in comparison to the control and Hx group.

Histopathology & Immunohistochemistry

Histrogical sections of SU5416 treated rat lungs had airspace enlargement. There were no evidence of plexiform lesions within pulmonary arterioles, a morphologic hallmark of pulmonary arterial hypertension of group 1 PH. In the lung blood vessel of SUHx group, the medial wall hypertrophy was mainly observed.

Protein expression

The expression of caspase3 was significantly higher in the SUHx group than in the control and the Hx group. The expression of eNOS in the lungs was significantly decreased in the SUHx group.

Messenger RNA expression

We confirmed that SUHx group was in fact characterized by the higher expression of inflammatory molecules (IL6, TNFα. MCP1) in the lungs.On the other hand, the expression of VEGF A was decreased in SUHx group.

Discussion

- As already reported, SU5416-induced emphysema was characterized by the higher expression of caspase3 in the lungs [J Clin Invest 2000;11:1311-1319].
- Under the influence of chronic hypoxia exposure, We confirmed that the SU5416 treated rat caused pulmonary hypertension that was not severe like group1. The lack of the plexiform lesions represents the pathological characteristic of group 3 PH.

Conclusion

- Our findings suggest that exposure the rats treated with VEGF receptor inhibitor to chronic hypoxia induced PH that was highly associated with the development of emphysema. This novel animal model would be mimic of the pathophysiological changes in human group 3 PH.

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