Assessment of the Pulmonary Circulation in Health and Disease using Synchrotron Radiation Microangiography

Pulmonary arterial hypertension (PAH) is characterized by the irreversible remodeling and medial thickening of small peripheral pulmonary arteries (<500 μm), causing a reduction in vessel internal diameter (ID) and, consequently, an increase in pulmonary vascular resistance. The subsequent increase in the workload of the heart enhances the risk of heart failure and is, therefore, closely associated with an increased mortality.

Despite decades of research, the structural and functional changes of the pulmonary vasculature during the pathogenesis of pulmonary arterial hypertension (PAH) still remain to be fully elucidated. One of the limitations in understanding the pathology of the lung has been the inability to clearly visualize blood flow within the pulmonary vascular bed. Conventional angiography systems, which have previously been used for visualizing pulmonary vessels, have considerable resolution limitations so that protocols are limited to i) open-chest models that expose the lung, and ii) images of vessels no smaller than 200 μm.

In the last decade, technological advances in microangiography have utilized monochromatic synchrotron radiation (SR) as a powerful X-ray source, providing the ability to study microvessels of various organs in unprecedented detail. In contrast to conventional systems, SR is characterized by high brilliance and extreme collimation, allowing enhanced sensitivity to contrast material and superior image quality. Therefore, we have utilized the high definition X-ray source of SR at **BL28B2** beamline to visualize the pulmonary microvessels within an intact-chest rat model - a technique not previously possible with conventional X-ray systems.

Rats were anesthetized and surgically prepared as previously described [2]. Rats were positioned in front of the beam pathway, so that the synchrotron beam passed, from anterior to posterior, through the rat thorax and ultimately to a biomedical imaging SATICON X-ray camera (Fig. 1). An iodinated contrast agent (Iomeron 350) was injected at high-speed (0.4 ml @ 0.4 ml/s) into the right ventricle so as to provide a concentrated dose of agent within the pulmonary vasculature to ensure optimal contrast of microvessels.

Using SR, we could clearly visualize pulmonary vessels, ranging from the main axial artery (~1200 μm) to the 4th generation of branching (~100-200 μm), in the left lung of an intact-chest rat (Fig. 2(a)). The smallest vessels that could be clearly measured were ~80 μm. This resolution is suitable for the purpose of the present study because the majority of vessels responsible for an increase in vascular resistance in pathological conditions generally have an ID between 50 μm and 300 μm [2-4].

Subsequently, we imaged the pulmonary vasculature of rats with PAH. PAH was induced by exposing rats to chronic hypoxia (10% O_2) for 4 weeks [1]. Compared to the normal lung, we observed significant structural and functional differences in the hypertensive lung. Importantly, PAH-rats had significantly fewer perfused 3rd and 4th generation vessels (9 and 16 vessels, respectively) compared to normal-rats (14 and 30 vessels, respectively) (Fig. 2(a) *vs.* 2(b)). This reduction in parallel blood flow in the hypertensive lung is the leading causal factor for the increase in pulmonary vascular resistance and, consequently, pulmonary arterial pressure.

The pulmonary vascular reversibly constricts in response to acute hypoxia. Therefore, we were able to assess functional dynamic changes in pulmonary reactivity. We observed that acute exposure to hypoxia (8% O_2 for 4 min) caused a significant decrease in the ID of all vessels less than 500 μm,

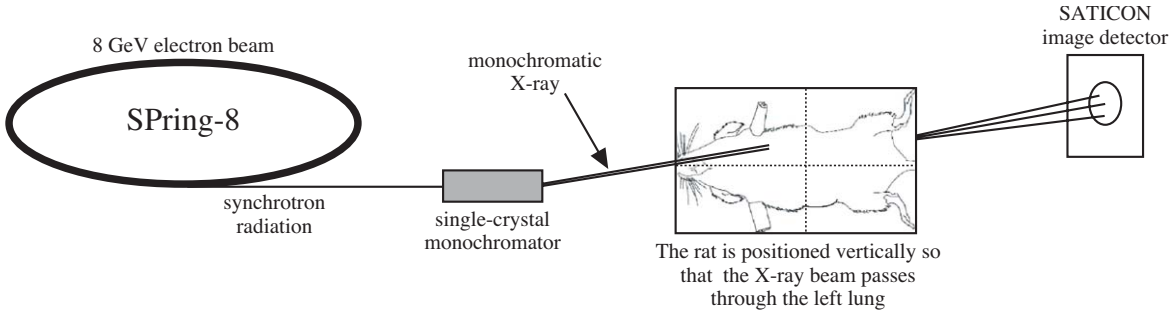

Fig. 1. A simplified schematic diagram showing the experimental set-up for pulmonary microangiography in a closed-chest rat model using SR.

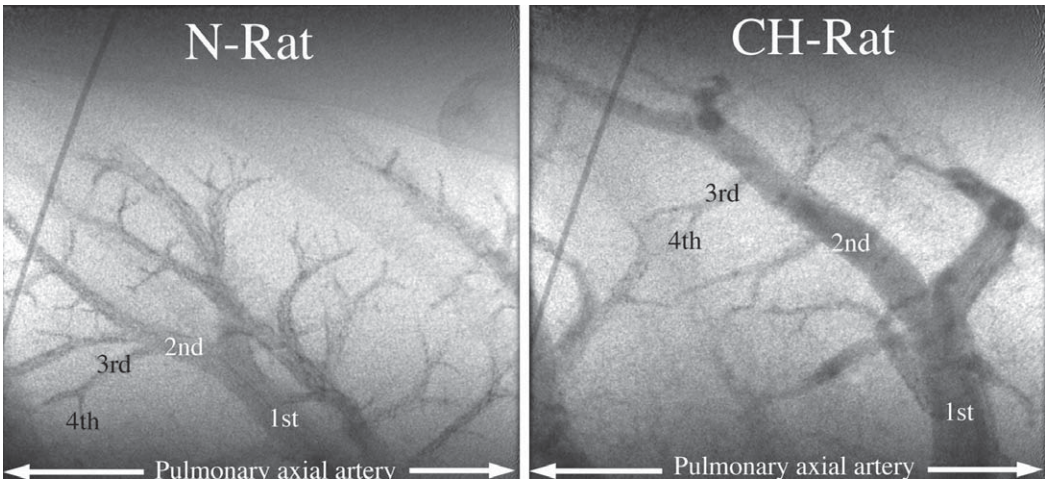

Fig. 2. Typical microangiogram images showing the branching pattern of small pulmonary arteries in **(a)** normal rats (N-Rat) and **(b)** hypertensive rats (CH-Rat). Pulmonary branches to the 4th generation from the left main axial artery (not in the image) were visible. The tungsten wire in the top left corner of each image is a reference of 100 μm diameter.

most noticeably in the 200-300 μm vessels (Fig. 3) - similar for both normal and PAH rats.

In summary, the use of SR provides a powerful tool for visualizing the pulmonary circulation in a closed-chest rat model. Furthermore, the high definition of SR permits detailed evaluation of the dynamic changes associated with acute pulmonary vasoconstriction and, more importantly, the gross structural changes in pulmonary vessel density of the hypertensive lung. Of particular importance, future use of SR will provide an effective method for assessing potential therapeutic treatments for PAH.

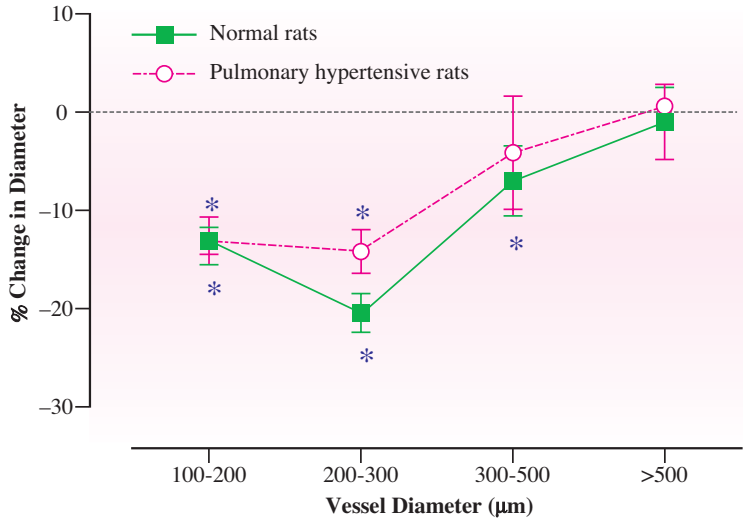

Fig. 3. The relationship between vessel size and the magnitude of pulmonary vasoconstriction (% decrease in vessel diameter) in response to acute hypoxia (8% O_2 for 4 min) in normal rats (n = 5) and pulmonary hypertensive rats (n = 5). * Significant vasoconstriction response to acute hypoxia ($P<0.05$).

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References

[1] *D.O. Schwenke, J.T. Pearson, K. Kangawa, K. Umetani and M. Shirai: J. Appl. Physiol.* ***104*** *(2008) 88.*
[2] D.O. Schwenke *et al.*: J. Appl. Physiol. **102** (2007) 787.
[3] R. Tokiya *et al.*: Acad. Radiol. **11** (2004) 1039.
[4] T. Yamashita *et al.*: Circ. J. **66** (2002) 1057.