# Studies on pathogenesis of acute thrombosis in Phenylhydrazine treated rat

Summary of Doctor Thesis

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Phenylhydrazine (PHZ) is used worldwide, mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. PHZ is well known for its ability to induce hemolysis via auto-oxidation of erythrocyte in animals including human. Several toxicities of PHZ has been reported in hematology and histopathology, e.g. decreased erythrocyte and hemoglobin, splenomegaly, erythrophagia and blown pigment deposition in various organs. On the other hand, thrombosis caused by PHZ has not been reported in the animal experiments. We could see a few cases of thrombosis in the patients treated with PHZ for polycythemia; however, the mechanism is unknown.

We found that a short-term administration of PHZ caused acute thrombosis in rat lung. In this study, we determined the hematological and histological changes of PHZ-treated rats to reveal the pathogenesis of the pulmonary thrombosis. In addition, we used gene expression profiling to provide a better understanding of biofunctional changes in lungs of PHZ-treated rats.

## 1. Acute pulmonary thrombosis and other histopathological findings in major organs of PHZ-treated rats (Second chapter)

To obtain the pathological reference data for blood or hematopoietic toxicity, we conducted a short-term repeated administration study of PHZ in young male SD rat. Macroscopically, PHZ-treated rats showed red or dark discoloration in all lobes of lungs and small white lesions are scattered. Microscopically, fibrinous thrombi were formed in alveolar capillaries coincided with the white lesions macroscopically observed. In the other organs, hemolysis-related changes such as erythrophagia, extramedullary hematopoiesis and brown pigment deposition were observed in the liver, kidney, heart and spleen. Thrombi were observed in the left cardiac auricle mainly in dead animals; however, no microthrombosis were observed in the organs except for the lung. Therefore, it is

considered that PHZ caused acute thrombosis specific to the alveolar capillaries in rats.

## 2. The hematological and histopathological time course of change in PHZ-treated rats (Third chapter)

To investigate the pathogenesis of the acute pulmonary thrombosis in PHZ-treated rats, we evaluated the hematological and histopathological time course. As a result, the earliest change except anemia that preceded thrombus formation was congestion (i.e. accumulation of deformed erythrocyte). In addition, abnormality in coagulation parameters and limited endothelial injury in the alveolar capillaries were also observed accompanied by diffuse thrombus formation. Applying these changes to the three major causes of thrombus formation as follows: 1) endothelial injury, 2) stasis or turbulence of blood flow, and 3) blood hypercoagulability; the trigger for acute pulmonary thrombosis in PHZ-treated rats was considered to be regional stasis (Factor 2). Endothelial injury (Factor 1) and blood hypercoagulability (Factor 3) was considered to accelerate thrombus formation.

## 3. The time course change of mRNA in the lungs of PHZ-treated rats (Forth chapter)

As mentioned above, it was revealed that endothelial injury contributed to acceleration of thrombosis; however, the potential of endothelial dysfunction remains unclear. Therefore, we evaluated the expression changes of well-known thrombosis-related genes in endothelium in the lungs of PHZ-treated rats. In addition, we used gene expression profiling and Gene ontology analysis to provide a better understanding of the pathogenesis of the thrombosis. As a result, some of the thrombosis-related genes we examined were significantly changed mainly during thrombus formation and the

balance between coagulation and fibrinolysis was considered to be inclined to pro-coagulant force. This change would be contribute to the development of the pulmonary thrombosis in PHZ-treated rats. The gene expression analysis showed that inflammation/immune response was significantly and continuously induced in the lungs of PHZ-treated rats from the early phase of treatment. It has been reported that inflammatory response causes endothelial dysfunction and interact with blood coagulation. Therefore, inflammatory condition in lungs of PHZ-treated rats would be play a role in acute thrombosis.

To summarize the findings of our studies and other relevant reports, we propose a putative mechanism for acute pulmonary thrombosis in PHZ-treated rats (figure below). PHZ affected erythrocytes and might cause various types of disruption, including loss of deformability and morphological alteration, which are attributable to regional stasis, endothelial dysfunction and systemic hemostatic disruption (i.e. blood hypercoagulability). Inflammatory condition in the lung provoked from early phase might induce the endothelial dysfunction. Considering the impact and the onset of the events observed in our study, regional stasis could serve as a trigger, and subsequent endothelial dysfunction in the lungs and blood hypercoagulability would be important contributors to acute thrombosis in the lungs of PHZ-treated rats.