# 学 位 論 文 の 要 旨

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学　位　論　文　名　Effects of the *Prdx2* Depletion on Blood Pressure and Life Span in Spontaneously Hypertensive Rats

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## 論 文 内 容 の 要 旨

### INTRODUCTION

The stroke-prone spontaneously hypertensive rat (SHRSP) is known to be highly susceptible to cerebral stroke under salt loading. As this strain shows a greater level of oxidative stress than the stoke-resistant spontaneously hypertensive rat (SHR), oxidative stress may play an important role in the stroke-susceptibility in SHRSP. Oxidative stress is defined as an imbalance between the production of free radicals and the antioxidant defense system in the body. Previous studies have indicated that oxidative stress is involved in the pathogenesis of cardiovascular diseases including hypertension and hypertensive organ damages. We therefore hypothesized that chronic increase of oxidative stress in SHR would accelerate hypertension and hypertensive organ damages such as cerebral stroke and renal failure. To examine this possibility, we constructed a ‘knock-out’ SHR in which *Prdx2* (a gene coding an antioxidant enzyme) was depleted by the genome editing technology using CRISPR/CAS9. In this study, we found that the *Prdx2*-depletion caused shorter life span as well as modest blood pressure (BP) increase in SHR through increase in oxidative stress although acceleration of stroke and hypertensive renal injury was not apparent.

### MATERIALS AND METHODS

All experimental protocols of this study except genome editing using CRISPR/CAS9 were approved by the local committee of animal research in Shimane University. The procedure of genome editing using CRISPR/Cas9 was approved by the local committee of animal research in Kyoto University.

A knockout SHR (KO-SHR) with a 7 bp-deletion in the *Prdx2* gene was constructed using CRISPR/CAS9. Lack of the PRDX2 protein expression was confirmed by a western blotting and the depletion of *Prdx2* mRNA expression was checked by RT-PCR analysis. Male SHR and KO-SHR at 8 weeks of age were used in the experiments. Rats were fed with stroke-permissive (SP) diet (Funabashi Farm Co., Ltd., Chiba, Japan). After BP measurement, salt-loading was started using 1% NaCl in drinking water for 8 weeks. Control rats were fed with plain water. Food and water intake were monitored every day whereas body weight (BW) and BP (by tail-cuff method) were measured at every two weeks. In some experiments, 24-hrs urine, serum and organs (kidneys, brain and heart) were collected after 8 weeks of salt-loading for biochemical, gene expression and histological studies. BP measurement was performed using the radio telemetry system as well. Survival of SHR and KO-SHR was examined up to 90 days of salt loading in a separate experiment. Oxidative stress was evaluated by measurement of urinary isoprostane and DHE staining of the brainstem and kidney tissues. Urinary protein, blood urea nitrogen (BUN) and serum creatinine were quantified using a commercial kit. Expression of genetic markers for renal fibrosis and tubular injury were evaluated by RT-PCR. Histological analysis of renal and cardiac injury was done on digital images of tissue samples stained by Haematoxylin & Eosin (HE) and Azan method. Statistical analysis was performed using the Student's *t* test and by the $\chi^2$ test. $p < 0.05$ was considered to be significant.

## RESULTS AND DISCUSSIONS

Oxidative stress evaluated by urinary isoprostane was greater both in SHR and KO-SHR under 1% salt loading when compared with water treated control rats. Furthermore, the level of isoprostane in urine was significantly higher in KO-SHR than in SHR both at baseline and salt-loaded condition, indicating that the knockout of *Prdx2* successfully augmented oxidative stress level in this hypertensive model. Similarly, the results of DHE staining showed that production of reactive oxygen species (ROS) in the kidney was significantly greater in KO-SHR than in SHR. Systolic Blood Pressure (SBP) measured by the tail-cuff method was significantly higher in the KO-SHR (170±12 and 152±12 mmHg for KO-SHR and SHR at 10 weeks old, respectively) under baseline condition. Salt treatment significantly increased BP both in SHR and KO-SHR but the difference between them was abolished. This was further confirmed by the telemetry measurement of BP. Telemetry method showed the result of BP consistent with the observation by the tail-cuff method. In spite of no difference in BP under salt-loading, life span in the KO-SHR was significantly reduced than in SHR (the median of life span was 52 and 81 days for KO-SHR and SHR, respectively. $p=0.049$ by the log-rank test). MRI and macroscopic observation of dissected brains of died rats were done to check whether the rats had cerebral stroke. Unexpectedly, however, no pathological changes indicating the onset of cerebral stroke

were found both in KO- SHR and SHR, suggesting that increase in oxidative stress did not exacerbate stroke susceptibility in SHR.

Different studies have shown that higher levels of oxidative stress have effect on renal and cardiac pathophysiology. In this study kidney and heart weights in SHR and KO-SHR were increased significantly under salt treatment but the differences between them were not significant both under control and salt-loaded condition. In an analogous manner, severity of hypertensive renal and cardiac injury did not differ significantly between the two strains in terms of prevalence of sclerotic glomeruli and relative area of renal and cardiac fibrosis but a significant increase in the prevalence of total (completely + partially) sclerotic glomeruli was observed in the KO-SHR under salt treatment. In consistent with that, expression of genetic markers for renal fibrosis ( $\alpha$ -*Sma, Tgf-*$\beta$*, Col1a1 and Col4a1*) and for tubular injury (*Kim-1*) did not differ significantly between the KO-SHR and SHR. Further, salt-loading increased urinary protein excretion, blood urea nitrogen (BUN) level and serum creatinine both in SHR and KO-SHR but neither urinary protein excretion nor BUN and serum creatinine differed between the two strains. These observations indicated that the *Prdx2* depletion did not deteriorate hypertensive organ damages in SHR in spite of significant increase in oxidative stress.

## CONCLUSION

In conclusion, we established a *Prdx2* KO-SHR, which showed significant increase in baseline BP and a shorter life span under salt-loading probably through greater oxidative stress. At the moment, the pathological mechanisms of the reduced life span were unclear as clear effects of the *Prdx2* depletion on cerebral, renal and cardiac pathology were identified. Further studies are required on pathophysiological pathways how the increased oxidative stress reduced life span in the KO-SHR.