学 位 論 文 の 要 旨

氏名 DAVIS LEONCE NGARASHI

学 位 論 文 名	Dual Inhibition of NADPH Oxidases and Xanthine Oxidase Potently Prevents Salt-Induced Stroke in Stroke-Prone Spontaneously Hypertensive Rats
発 表 雑 誌 名 （巻，初頁～終頁，年）	Hypertension Research (*In press*)
著 者 名	Davis Ngarashi, Koichi Fujikawa, Mohammed Zubaerul Ferdaus, Hasan MD Zahid, Hiroki Ohara, Toru Nabika

論 文 内 容 の 要 旨

INTRODUCTION

Several experimental studies reported that increase of oxidative stress was one of important mechanisms underlying stroke. Further, it was proposed that NADPH oxidases (NOXs) played a major role in the regulation of oxidative stress. As the stroke-prone spontaneously hypertensive rat (SHRSP) is well-characterized with its unique stroke susceptibility and a high level of oxidative stress *in vivo*, new insights regarding roles of oxidative stress in the pathogenesis of stroke may be obtained through studies on this rat model. Hence, in the present study, we examined effects of genetic depletion of *P22phox*, an essential subunit of the NOX complex, on salt-induced stroke in SHRSP. We hypothesized that reduction of the NOX activity by depletion of *P22phox* would prevent cerebral stroke. In addition, we examined three different pharmacological strategies to reduce oxidative stress in SHRSP; scavenging reactive oxygen species (ROS) with Tempol, reducing mitochondrial ROS production with coenzyme Q10 (CoQ10), and inhibiting the xanthine oxidase (XO) with febuxostat.

In this report, we showed that depletion of the NOX activity alone did not prevent stroke in SHRSP. Instead, it was necessary to add one of the three reagents described above to achieve the prevention. However, it was of note that administration of febuxostat alone was significantly effective to reduce stroke in SHRSP. The physiological significance of these observations was

discussed.

MATERIAL AND METHODS

The *P22phox*-depleted congenic SHRSP (called SP.MES) and SHRSP were employed in this study. Experiments were started on 8-weeks-old male rats. In the first week, rats were fed with regular drinking water with or without Tempol (3 mM in drinking water), febuxostat (30 mg/L in drinking water) or CoQ10 (200 mg/L in drinking water). To evaluate stroke latency, drinking water was then switched to 1% salt water with or without one of the three reagents above. Rats were then closely checked every day for suggestive signs of stroke where MRI was also used for confirmation. Twenty-four-hours urine collection was done by using metabolic cages before salt-loading (i.e., after 1-week treatment with the three antioxidant reagents) and after two weeks treatment with the reagents + 1% salt. At the end of experiments, the rats were sacrificed and kidneys and serum samples were harvested. The urinary isoprostane, urinary protein and serum uric acid were quantified using standard methods available. Blood pressure (BP) was measured by the tail-cuff and the radio-telemetry methods. To evaluate renal damage, we examined incidence of sclerotic glomeruli on H&E-stained histological specimen. Statistical analysis was performed using the Student's t test, Chi-square test and One-way ANOVA when appropriate. All experimental protocols of this study were approved by the local committee of animal research in Shimane University.

RESULTS AND DISCUSSION

Under salt-loading, *P22phox*-depletion did not ameliorate oxidative stress nor the incidence of stroke in SP.MES although it exhibited a significantly lower number of sclerotic glomeruli as well as lower BP and urinary protein level both under baseline and salt-loaded conditions when compared with SHRSP. On the other hand, administration of different antioxidant reagents (Tempol, febuxostat and CoQ10) to SP.MES reduced stroke incidence as well as oxidative stress under salt-loading, suggesting that, although oxidative stress *per se* was an important factor in the pathogenesis of stroke, inhibition of NOX was not enough to prevent stroke in SHRSP. The significance of oxidative stress in cardiovascular pathology in SHRSP was further supported by correlation analyses, in which significant correlations of oxidative stress with BP, stroke latency and urinary protein level was observed under salt-loading. Interestingly, when effects of each of the antioxidant reagents on cardiovascular phenotypes were examined in SHRSP, only febuxostat (not Tempol nor CoQ10) elicited significant reduction of salt-induced stroke, oxidative stress and BP. This result implied that XO was a major generator of oxidative

stress in SHRSP, which influenced BP and stroke incidence.

In contrast, the examination of renal pathology in SP.MES and SHRSP revealed that inhibition of NOX by *P22phox* depletion was sufficient to ameliorate salt-induced renal injury in SHRSP, which was further improved by addition of Tempol. This suggested that, in SHRSP, the kidney was more vulnerable to oxidative stress than was the brain in terms of stroke susceptibility.

CONCLUSION

In conclusion, our study indicated that NOX was not a major source of oxidative stress in SHRSP. Instead, ROS from multiple sources affected stroke-susceptibility and other hypertensive disorders in salt-loaded SHRSP. In addition, XO may be a promising target to improve hypertensive organ damages in SHRSP. Further studies on the role of XO in cardiovascular diseases are warranted.