学 位 論 文 の 要 旨

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学 位 論 文 名	Evaluation of Pathological Association between Stroke-related QTL and Salt-induced Renal Injury in Stroke-Prone Spontaneously Hypertensive Rat.
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論 文 内 容 の 要 旨

INTRODUCTION

The stroke-prone spontaneously hypertensive rat (SHRSP) is a good genetic model for hypertension and hypertension-related organ damages such as cerebral stroke. Hence, it was proposed that studying genetic mechanisms of stroke in SHRSP was useful in exploration of the genetic susceptibility to stroke in humans. In this context, we performed a genetic study and identified two major quantitative trait loci (QTLs) for stroke latency on chromosome 1 and 18 in SHRSP and confirmed their effects in reciprocal congenic strains. In short, congenic strains having SHRSP-derived QTL fragments of chromosome 1 and 18 on SHR background (abbreviated as Rp1.0 and Rp18.0, respectively) showed a higher incidence of stroke when compared with SHR. Furthermore, several observations suggested that SHRSP exhibited severe renal damage whereas SHR shows milder or even absence of it. We hypothesized that, if the susceptibility to hypertensive renal damage was influenced by the QTLs for stroke on chromosome 1 and/or 18, it might be helpful to identify the responsible genes and to understand their functions. In this study, we therefore examined whether the severity of salt-induced renal damage would differ among the three strains, i.e., Rp1.0, Rp18.0 and SHR.

MATERIAL AND METHODS

In this study, six rats were used in each group. Experiments were started when rats were

twelve weeks of age. After measuring baseline blood pressure and body weight, 1% salt solution was loaded and stroke permissive diet was fed for four weeks. Blood pressure and body weight were monitored weekly and twenty-four-hour urine (using a metabolic cage) was collected every two weeks. Urine samples were kept frozen for further biochemical assay. Blood pressure was measured by the tail-cuff method. After completion of the experiments, rats were deeply anesthetized, perfused with saline and two kidneys were collected. The left kidney was preserved for histology analysis and the right kidney was dissected and stored frozen state to perform RNA extraction later. Urinary isoprostane was measured using an ELISA kit and urinary protein level was determined by the BCA kit according to manufacturer's instruction. To evaluate morphological changes, we examined glomerulosclerosis and fibrosis in the left kidney. About three hundred glomeruli were examined for each rat, and, according to the severity of damage, glomeruli were categorized into three groups, i.e., no, partially and completely sclerosis. To evaluate fibrosis, relative fibrotic area was compared among the strains on Azan-stained specimens using the NIH Image J. Further, we evaluated the expression of three fibrotic markers (*Col1α-1*, *Tgf- β* and *α-Sma*) and a tubular injury marker *Kim-1* by the quantitative RT-PCR method in renal cortical tissue. Relative amount of mRNA of those markers were calculated against β–actin mRNA as a control. Statistical significance was tested either by $\chi 2$ test or Student's t-test. The research protocol was approved by the local ethical committee of animal research in Shimane University.

RESULTS AND DISCUSSION

During salt-loading, blood pressure was gradually increased in all the strains. Morphological assessment revealed that Rp18.0 showed a greater incidence of sclerotic glomeruli than that in Rp1.0 and SHR. Fibrotic area in the kidney tended to be greater in this strain as well. Consistently to those observations, the urinary protein level was increased in Rp18.0 under the salt-loading. Evaluation of expression of the genetic markers for renal fibrosis, we found that *Col1α-1* expression was significantly higher in Rp18.0 than in the other two strains. *Tgf- β* was also tended to be higher in Rp18.0. In accordance with that, a strong correlation of the two genetic markers, *Col1α-1* and *Tgf- β,* with the relative fibrotic area and the incidence of glomerulosclerosis was observed. The urinary isoprostane level was increased in all the strains during salt-loading and Rp18.0 showed a significantly greater level of isoprostane after four weeks of salt-loading.

In this study, we found that Rp18.0 was more susceptible to salt-induced renal damage than Rp1.0 and SHR. We suggested that this phenomenon was blood pressure independent because blood pressure of Rp18.0 was not greater than that of Rp1.0 and SHR. We suggested a

hypothesis to interpret the results; genes in the congenic region responsible for stroke might also be responsible for the renal damage. This congenic region on chromosome 18 might affect stroke and the renal damage as parallel events. We showed that salt-loading increased oxidative stress more in Rp18.0, which might worsen glomerulosclerosis and renal fibrosis. It is attractive to hypothesize that oxidative stress was increased in the brain in the salt-loaded Rp18.0 as well, which might facilitate cerebral stroke.

CONCLUSION

We found that congenic rat with an SHRSP-derived fragment of chromosome 18 showed more severe hypertensive renal injury with no significant increase in blood pressure. As a higher urinary isoprostane level was found in this congenic rat, we proposed a possibility that a gene or genes in the congenic region of chromosome 18 might provoke both renal damage and stroke through increase of oxidative stress. Pathological mechanisms how the genes in the congenic region increase oxidative stress in the salt-loaded condition, and how oxidative stress deteriorates stroke incidence and renal damage should be explored in future studies.