学 位 論 文 の 要 旨

氏名　AMELIA FERDAUS SARA

学 位 論 文 名　NAD^+ Deficiency Plays Essential Roles in the Hyperuricemia of Stroke-Prone Spontaneously Hypertensive Rat via Xanthine Dehydrogenase to Xanthine Oxidase Conversion

発 表 雑 誌 名　Biochemical and Biophysical Research Communications

（巻，初頁～終頁，年）(in press, 2024)

著 者 名　Sara Amelia FERDAUS, Hiroki OHARA, Hiroyuki MATSUO, Kohei KAWAKAMI, Fumihiko TAKEUCHI, Koichi FUJIKAWA, Emi Kawakita, Norihiro KATO, Toru NABIKA, Keizo KANASAKI

論 文 内 容 の 要 旨

INTRODUCTION

Oxidative stress contributes to the pathophysiology of hypertension and stroke. Several evidence have reported the significant role of oxidative stress in the stroke-prone spontaneously hypertensive rat (SHRSP), an appropriate genetic rat model to study hypertension and stroke. Xanthine oxidoreductase (XOR), one of the important regulators of oxidative stress, yields uric acid (UA) as the final product of purine metabolism in humans. High serum UA level has been associated with the pathophysiology of hypertension and stroke as well. In the previous study, XOR was found to be mainly responsible for stroke susceptibility in SHRSP suggesting that hyperuricemia had a significant role in this rat model. In the present study, we aimed to investigate the mechanistic role of XOR causing hyperuricemia in SHRSP. XOR has two interconvertible forms, the dehydrogenase form (XDH) and the oxidase form (XO) which is converted from XDH through either proteolytic cleavage or disulfide bond formation in the

enzyme. The XO form losing affinity towards NAD^+ uses oxygen as the cofactor while metabolizing purine to UA and gives reactive oxygen species (ROS) as the byproduct, in contrast to the XDH form. Thus, we hypothesized that excess conversion to XO caused the higher UA level and ROS in SHRSP. In the current report, we evaluated the XO/XDH conversion as well as the role NAD^+ deficiency in SHRSP.

MATERIALS AND METHODS

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University. Serum UA level was measured at 10^{th} week of age of male SHRSP and compared with that of Wister-Kyoto rats (WKY) as the control group. Liver lysate samples were used to assess XO activity and Uricase activity using respective commercial enzymatic assay kits. Meanwhile, total XOR protein expression was measured by western blotting analysis. NAD^+ level and NADH level in the liver lysate were measured using commercial assay kit assay. Based on these results, 8 weeks old male SHRSP rats were randomly divided into three groups provided with plain water (1) control (Cont) group, (2) febuxostat (Febx) group and (3) b-nicotinamaide mono nucleotide (NMN) group dissolved in the regular drinking water for 2 weeks. The body weights (BW) and systolic blood pressure (SBP) were recorded before and after the treatment. After their sacrifice at 10 weeks of age, similar experiments were conducted.

RESULTS AND DISCUSSION

Serum UA level and XO activity were found significantly higher in SHRSP than WKY. The uricase activity did not differ significantly implying that uricase did not contribute to the different levels of serum UA in the two strains. As the total XOR protein expression was not significantly different between the two strains being showed only at 150 kD size in western blotting analysis suggesting that no proteolytic cleavage occurred in the conversion from XDH to XO. While NAD^+ level was significantly lower in the SHRSP indicating that low NAD^+ level promoted the reversible conversion of XO from XDH in this strain. NMN supplementation as

well Febx treatment for 2 weeks did not exert any significant effect on BW and SBP of SHRSP. The result of Febx treatment in the present study for BP was inconsistent with the previous study. This may be due to the difference in experimental designs, that is, with or without salt loading. It would be necessary to increase the sample number and/or to take a longer experimental period to obtain a significant result.

As expected, NMN supplementation increased the NAD^+ level and reduced serum UA and liver XO activity significantly in SHRSP. Expectedly, Febx significantly reduced serum UA and liver XO activity but no significant increase in the NAD^+ level. No changes in the total XOR protein expression were found in the NMN or Febx treatment. These results supported that a deficiency in NAD^+ level promoted the conversion of XDH to XO in SHRSP which resulted in high serum UA which could be ameliorated by increasing the NAD^+ level via NMN supplementation, the precursor of NAD^+.

CONCLUSION

The present study showed that XO activity to be significantly higher in SHRSP which could be ameliorated by NMN supplementation. Although the mechanism behind high XO activity in SHRSP was not thoroughly clarified but the key role of the NAD^+ level on the reversible conversion from XDH to XO was reflected by the result of NMN supplementation. Overall, our findings suggested the potential significance of NMN supplementation inhibiting XO activity as a possible therapeutic approach to prevent cardiovascular complications associated with oxidative stress and hyperuricemia in humans.