学 位 論 文 の 要 旨

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学 位 論 文 名　Effect of High-fat Diet on Cerebral Pathological Changes of Cerebral Small Vessel Disease in SHR/SP Rats

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論 文 内 容 の 要 旨

INTRODUCTION

Cerebral small vessel diseases (CSVD) encompass various chronic neurological conditions resulting from damage to small vessels in the brain's cortex, white matter (WM), and subcortical areas. Clinical manifestations include lacunar strokes, intracerebral hemorrhages, and observable WM alterations on MRI scans. Symptoms vary based on the location and extent of damage, including cognitive impairment, gait disturbances, mood changes, and focal neurological deficits. Pathologically, CSVD includes arteriosclerosis, cerebral amyloid angiopathy (CAA), genetic CSVDs (CADASIL and CARASIL), inflammatory variations, and others. Arteriosclerosis, prevalent in the elderly, involves sclerotic changes leading to impaired autoregulation and reduced perfusion.

Hypertension is a primary cause, with risk factors including dyslipidemia, diabetes, smoking, and a Western-style diet high in saturated fat. Obesity-induced CSVD often exhibits lacunae without prominent white matter hyperintensities (WMH). Hypertension, obesity, and dyslipidemia often coexist, impacting CSVD with varying effects. However, the exact connection between these factors and molecular changes in CSVD is not well-known. In this study, we hypothesized that a combination of risk factors, such as hypertension and a high-fat diet (HFD), might exacerbate CSVD differently than a single risk factor.

To investigate this, we utilized a hypertensive CSVD model exposed to HFD, specifically male spontaneously hypertensive/stroke-prone (SHR/SP) rats. The SHR/SP model is suitable for studying hypertension-induced CSVD, exhibiting small vessel sclerosis, blood-brain barrier

changes, and vessel degeneration. Additionally, we included normotensive male Wistar-Kyoto rats (WKY) in our study to assess the independent effects of HFD on CSVD. Our findings indicate that HFD indeed increases CSVD in a hypertension-independent manner. Thus, our study not only demonstrated that HFD can contribute to cerebrovascular disease but also emphasized its role in exacerbating the disease process in conditions without hypertension, providing a sound scientific basis for further treatment and intervention.

MATERIALS AND METHODS

We used male 12-week-old WKY, SHR, and SHR/SP rats divided fed 8 weeks with a normal diet (ND) or with HFD (60% lard). They were then divided into 6 experimental groups, including the ND groups (ND-WKY, ND-SHR, and ND-SHR/SP) and the HFD groups (HFD-WKY, HFD-SHR, and HFD-SHR/SP), with approximately 8 rats in each group, totaling 48 animals. The body weight of each group was measured weekly throughout the experiment. Subsequently, 4 rats in each of the HFD groups (HFD-WKY, HFD-SHR, and HFD-SHR/SP) were subjected to blood pressure and brain magnetic resonance imaging (MRI) before and after 8 weeks of HFD feeding in the HFD groups, respectively. One day after the brain MRI examination, brain tissues were taken from all groups of rats to observe pathological changes.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (approval number: IZ2-96).

RESULTS AND DISCUSSION

We first observed that the body weight of WKY, SHR, and SHR/SP rats increased gradually on a weekly basis, with a significant weight gain in all rat strains fed with HFD compared to the ND groups. Meanwhile, the rate of body weight increase in HFD-SHR/SP rats was lower than that in HFD-WKY and HFD-SHR rats. Blood pressure elevation was observed in SHR and SHR/SP rats compared to WKY before HFD, but after 8 weeks of HFD, only WKY rats showed an increase in blood pressure. Subsequently, we focused on observing the pathological changes in the brains of WKY, SHR, and SHR/SP rats under normal diet and high-fat diet conditions. Under hypertension alone, both SHR and SHR/SP rats displayed CSVD pathological changes, including reduced BBB tight junction protein claudin 5 expression, vessel leakage, elevated hypoxia factor HIF-1α, increased neuroinflammation markers (Iba-1, GFAP), and oxidative stress marker Gp91-phox in various brain regions. HFD exposure revealed specific pathological changes and exacerbated existing ones, contributing to the worsening of CSVD. Particularly, HFD induced increased vascular basement membrane protein collagen IV expression, unaffected by hypertension alone. The corpus callosum region in SHR/SP rats exhibited the greatest increase in HIF-1α expression and pronounced vascular leakage under HFD. Furthermore, HFD further intensified the neuroinflammatory response and aggravated oxidative stress in the intracerebral vasculature of SHR/SP rats across three regions. Interestingly,

HFD exacerbated the reduction of claudin 5 only in the vasculature of normotensive WKY rats and hypertensive SHR rats. KB staining results highlighted that HFD aggravated severe myelin loss in the corpus callosum region of SHR/SP rats. These changes led to a cumulative CSVD pathological effect, evident in the form of T2 WMH in the corpus callosum region of SHR/SP rats following HFD feeding.

The present study investigated the accumulative effect of HFD added upon hypertension overload in the CSVD pathology of SHR and SHR/SP rats. It is known that a high fat-containing diet, especially saturated fat, and hypertension can affect peripheral vessel health. In this study, we demonstrated that HFD can affect cerebral small vessels independently, especially vessel basement membrane. Moreover, HFD can augment hypertension-induced effects causing pathological changes similar to CSVD including BBB disruption, changes of vessel basement membrane, and increased neuroinflammation, vascular hypoxia and oxidative stress. WMH in T2-weighted MRI and decreased vessel integrity are considered diagnostic features of CSVD, especially hypertension-induced CSVD in humans. In the case of our study, HFD affects both of these features, especially in severely hypertensive SHR/SP rats. Also, pathological changes like hypertension-induced vessel leakage were increased by HFD across the different brain regions in all rat strains. Particularly, extensive vessel leakage was observed in the corpus callosum region of hypertensive SHR/SP rats fed with HFD. Although we observed that the vessel leakage was increased in hypertensive SHR rats, we did not find WM, cortical, or striatum hyperintensity even after HFD feeding. However, the SHR/SP rats have a more pronounced degree of hypertension compared to SHR rats. This suggested the possibility that the cumulative effects of hypertension and HFD on WM changes are greater in SHR/SP causing the change visible early, while similar changes potentially develop in SHR rats at a later time. Importantly, HFD also induced leakage in normotensive WKY rats, indicating it could be an independent risk for cerebral vessels. Hence, along with hypertension, dietary interventions might also need to be considered for the management of CSVD, and overall, the health of cerebral small vessels. Such findings contribute to a better understanding of the complex interplay between metabolic factors and hypertensive conditions in maintaining cerebrovascular and white matter health and help to formulate improved therapeutic management for multiple risk factors induced CSVD in human.

CONCLUSION

In conclusion, our study demonstrates that HFD, in combined with hypertension, intensifies cerebral pathological alterations in CSVD rats. This exacerbation involves increased oxidative stress and HIF-1α in cerebral vessels, triggering neuroinflammation, vascular basement membrane remodeling, IgG leakage, and ultimately white matter damage.