学 位 論 文 の 要 旨

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学 位 論 文 名　Apolipoprotein E – Depletion Accelerates Arterial Fat Deposition in the Spontaneously Hypertensive Rat

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論 文 内 容 の 要 旨

INTRODUCTION

Atherosclerosis is one of the most prevalent arterial diseases that causes severe cardiovascular events such as myocardial and cerebral infarction. A number of studies, both basic and clinical, were performed to clarify pathophysiological mechanisms of atherosclerosis to prevent severe cardiovascular events. Among them, it was of note that *apolipoprotein E (ApoE)*-depleted mice was developed in 1992, which has made tremendous contribution in basic studies of atherosclerosis. However, as *ApoE*-depleted mice had several limitations, it is worth to establish another model for atherosclerosis. Rats are preferred in some biological studies because of their larger body size suitable for physiological experiments. In addition, it is a great advantage that rats have genetic models for hypertension. Hypertension is another important cardiovascular disease widely seen in the world. The two vascular diseases, i.e., hypertension and atherosclerosis, were often observed in one patient and their combination was thought to deteriorate arterial damages. We therefore planned to establish a model for combined hypertension and atherosclerosis in rats to study effects of interaction between hypertension and atherosclerosis on the vasculature in detail. In this study, we employed the spontaneously hypertensive rat (SHR), the most popular genetic model for hypertension, to establish a model for combined hypertension and atherosclerosis through knocking out the *ApoE* gene by the genome editing strategy using CRISPR/Cas9.

Peroxiredoxin 2 (*Prdx2*) is one of key enzymes erasing reactive oxygen species in cells, and it was previously reported that *Prdx2*-depletion accelerated atheromatous lesions in *ApoE*-knockout mice. In this study, we therefore constructed *ApoE*-knockout and *ApoE-/Prdx2*-double knockout SHR ($SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$, respectively), and examine effects of those gene-knockouts on atherosclerosis in SHR.

MATERIALS AND METHODS

Male SHR and $SHR^{ApoE(-/-)}$ at 12-weeks old were used in the experiments. Body weight (BW) and systolic blood pressure (SBP) by the tail-cuff method (BP-98A, Softron, Tokyo, Japan) were measured at the start of experiments. Rats were divided into 4 groups that were fed either (i) the normal diet (MF) and water, (ii) MF and 1% salt water, (iii) high fat high cholesterol diet containing 1% cholesterol, 0.25% cholic acid, 15% cocoa butter (HFD) and water, or (iv) HFD and 1% salt water for 8 weeks. In the group (iii) and (iv), HFD was given to rats every second day (MF and HFD were given alternately) because continuous feeding of HFD induced sudden death of rats for an unknown reason. At the end of experiments, urine was collected for 24 hours with metabolic cages and then rats were fasted for 16 hours and euthanized under anesthesia using 3% of isoflurane to collect the aorta, the mesenteric artery and blood samples. Fat deposition on the arteries were quantified morphologically. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits. Urinary isoprostane (IsoP) was measured by ELISA. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University. (# IZ2-22)

RESULTS AND DISCUSSION

Even under MF, $SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$ showed a significantly greater level of TC and TG than those in SHR. In contrast, HDL-C was lower than that in SHR. Under HFD, greater increase of TC was observed in both $SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$. On the other hand, TG did not increase from the level in the rats under MF. In addition, HDL-C increased modestly in both $SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$ while no increase was observed in SHR. It was of note that *Prdx2*-depletion did not influence the profile significantly.

$SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$ had significantly greater fat deposition both in the aorta and the mesenteric artery under HFD when compared with SHR. No significant differences were observed in severity of fat deposition between $SHR^{ApoE(-/-)}$ and $SHR^{ApoE(-/-)Prdx2(-/-)}$. We expected that *Prdx2*-depletion in addition to hypertension would accelerate atherosclerosis in $SHR^{ApoE(-/-)Prdx2(-/-)}$, this study clearly showed that those were not enough to make severe atheromatous lesions in SHR, and that $SHR^{ApoE(-/-)Prdx2(-/-)}$ would not be a good model for

atherosclerosis observed in humans; as far as observed with a microscope, fat deposition was limited on the intimal surface of the aorta of SHR$^{ApoE(-/-)}$ and of SHR$^{ApoE(-/-)Prdx2(-/-)}$. This observation suggested that *ApoE*-depleted rats were resistant to atherosclerosis in general even if hypertension was added to promote endothelial damage. On the other hand, it is interesting that some studies successfully induced intimal thickening by implanting stents in the aorta or by keeping the rats under germ-free condition. These observations suggested that additional environmental factors modifying inflammatory and/or metabolic conditions in rats would be essential to induce mature atherosclerosis in rats. In addition, oxidative stress measured by urinary IsoP secretion did not differ significantly between SHR$^{ApoE(-/-)}$ and SHR$^{ApoE(-/-)Prdx2(-/-)}$ though IsoP tended to be greater under HFD. Finally, salt-loading accelerated the fat deposition both in the aorta and in the mesenteric artery not in SHR but in SHR$^{ApoE(-/-)}$. Urinary IsoP excretion increased in SHR$^{ApoE(-/-)}$ under salt-loading, suggesting that salt-loading accelerated the fat deposition through the increase in oxidative stress.

CONCLUSION

In conclusion, two knockout SHR strains, SHR$^{ApoE(-/-)}$ and SHR$^{ApoE(-/-)Prdx2(-/-)}$ were successfully established. These knockout SHRs, however, did not show severe atheromatous lesions even though they showed severe hypercholesterolemia and hypertension. Other environmental and/or genetic factors would be necessary to promote atherosclerosis in these rat models.