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専　　攻　　名	先進医療科学専攻
学位論文題名	Deletion of the apoCIII gene in knock-out rabbits enhances the catabolism of triglyceride-rich lipoproteins and protects against cholesterol diet-induced atherosclerosis (アポ CIII 遺伝子の欠損はノックアウトウサギの中性脂肪を富むリポ蛋白の異化を促進させ動脈硬化を抑制させる)
論文審査委員	委員長　教　授　中島　博之 委　員　講　師　髙野　勝弘 委　員　講　師　川端　健一

学位論文内容の要旨

Introduction

Apolipoprotein CIII (apoCIII) is a major component of plasma chylomicrons and very low density lipoproteins (VLDL), and is a minor component of high density lipoproteins (HDL) and was first described by Brown *et al.* 50 years ago. In humans, as a glycoprotein, apoCIII contains 79 amino acids with a molecular weight of 8.8 kDa and mainly expressed in the liver and lesser in small intestine. Normal plasma levels of apoCIII in healthy populations are at 7~12 mg/dL. In general, apoCIII mediates lipoprotein metabolism through inhibition of lipoprotein lipase (LPL) activity and thereby represses hepatic uptake of triglyceride-rich particles. Furthermore, some studies suggest that intracellular apoCIII promotes the assembly and secretion of triglyceride-rich VLDL particles from hepatic cells under lipid-rich conditions. Although the normal physiological functions are not fully understood, ample evidence from epidemiological, genetic and clinical studies has shown that increased plasma levels of apoCIII are directly associated with the development of hypertriglyceridemia and increases the risk of ischemic heart disease. Furthermore, large population genetic studies revealed that two naturally occurring point mutations in human apoCIII coding sequence, namely Ala23Thr and Lys58Glu have been shown to abolish the intracellular assembly and secretion of triglyceride-rich VLDL particles from hepatic cells. Humans deficient in apoCIII gene functions have been reported; however, it is still unknown whether these apoCIII deficient subjects

are susceptible to atherosclerosis or ischemic heart disease. On the other hand, many studies showed that therapeutic inhibition of apoCIII can alleviate human hypertriglyceridemia.

Functional roles of apoCIII in the development of hypertriglyceridemia was initially supported by the finding that overexpression of human apoCIII in transgenic mice and rabbits lead to a marked hypertriglyceridemia whereas apoCIII deficient mice exhibited hypotriglyceridemia. In spite of this, hypertriglyceridemia in transgenic mice even in the setting of apoE or LDL receptor deficient background was not atherogenic although one report showed mild atherogenic. In human studies, although high levels of plasma apoCIII was associated with high risk of ischemic heart disease, there is no evidence to show whether such association was causal or casual because it is still unknown whether apoCIII itself or hypertriglyceridemia or TRLs are directly atherogenic. In fact, it is generally believed that large particles of TRLs such as VLDL and chylomicrons are not atherogenic because their size does not allow them to penetrate into the arterial wall to initiate the lesion formation. Therefore, it is currently unknown whether inhibition of apoCIII can be used as a target for the treatment of atherosclerosis.

Objective

Apolipoprotein CIII (apoCIII) is a constituent of all lipoproteins except LDLs and mediates the metabolism of triglyceride (TG)-rich lipoproteins through inhibition of lipoprotein lipase activity. High levels of plasma apoCIII are correlated with the plasma TG levels and increase the cardiovascular risk. However, the pathophysiological functions of apoCIII *in vivo* have not been fully elucidated.

Approach and results

To examine the functional roles of apoCIII in lipoprotein metabolism and atherosclerosis, we generated apoCIII knockout (KO) rabbits using zinc finger nuclease technique. KO rabbits did not show any gross abnormalities. On a normal chow diet, apoCIII KO rabbits exhibited significantly lower plasma levels of TG than those of wild-type (WT) rabbits while total cholesterol and HDL-cholesterol levels were unchanged. Analysis of lipoproteins isolated by sequential gradient ultracentrifugation revealed that reduced plasma TG levels in KO rabbits were accompanied by 73% reduction of VLDLs and 57% reduction of intermediate-density lipoproteins (IDLs). In addition, KO rabbits showed faster clearance rate of intralipid emulsion than WT rabbits. On a cholesterol-rich diet, KO rabbits exhibited constantly lower levels of plasma total cholesterol and TG than WT rabbits, owing to a remarkable reduction of VLDLs, IDLs and LDLs. Aortic atherosclerosis areas were significantly reduced in KO rabbits compared with WT rabbits.

Conclusions

These results indicate that apoCIII deficiency facilitates TG-rich lipoprotein catabolism and therapeutic inhibition of apoCIII expression may become a novel means for the treatment of hyperlipidemia and atherosclerosis.

論文審査結果の要旨

（博士論文審査の結果の要旨）

The authors investigated the role of apolipoprotein CIII (apoCIII) in the lipoprotein metabolism. They focused apoCIII because it is a constituent of all lipoproteins except LDLs. ApoCIII mediates the metabolism of triglyceride (TG)-rich lipoproteins. High levels of plasma apoCIII increase the risk of cardiovascular disease. Thus apoCIII plays an important role in the development of atherosclerosis, however, the pathophysiology of apoCIII in vivo has not yet well elucidated.

They used apoCIII knockout rabbits. First, under a normal chew diet, the plasma levels of TG of apoCIII KO rabbits were lower than those of wild-type (WT) rabbits. They demonstrated that reduced plasma TG levels in KO rabbits were accompanied by 73% reduction of VLDLs and 57% reduction of IDLs. Knockout rabbits showed faster clearance rate of intralipid emulsion compared to WT rabbits. Secondly, under a cholesterol-rich diet, KO rabbits showed constantly lower levels not only of TG but also of plasma total cholesterol. Furthermore aortic atherosclerosis areas were reduced in KO rabbits compared to WT rabbits.

Those findings indicate that apoCIII deficiency facilitates TG-rich lipoprotein catabolism in vivo. The understanding of the mechanism of lipoprotein metabolism might open the door to the development of the novel approach to the more aggressive treatment of hyperlipidemia and atherosclerosis compared to conventional drug therapy.

The experimental procedures they took and the data obtained were reliable enough as a scientific paper. The doctoral dissertation defense committee considered this study contributes greatly to the development of treatment of hyperlipidemia. All committee members approve this paper is worth doctoral dissertation.