REMODELLING OF HIGH DENSITY LIPOPROTEINS BY PLASMA FACTORS

by

Hui-Qi LIANG

MB BS (China)
Master of Public Health

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Department of Medicine
The University of Adelaide
Adelaide, Australia

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TABLE OF CONTENTS

SUMMARY

The protective effect of high density lipoprotein (HDL) against coronary heart disease has stimulated a sustained interest in the metabolism and regulation of this lipoprotein fraction. Of particular interest has been the remodelling of HDL that takes place during their circulation in plasma. Several factors are known to operate in plasma to modify the lipid compositions of HDL. These include lecithin:cholesterol acyltransferase (LCAT) which catalyses the esterification of cholesterol in HDL; cholesteryl ester transfer protein (CETP), which transfers cholesteryl esters from HDL to other lipoproteins in exchange for either triglyceride or cholesteryl esters; hepatic lipase (HL), which hydrolyses HDL triglyceride and phospholipid; and phospholipid transfer protein (PLTP) which transfers phospholipid to HDL. These factors all have substantial effects on the concentration and composition of HDL lipids and on HDL particle size. However, there are little research done into the effect of these factors on apolipoprotein A-I (apo A-I), the main protein constituent of HDL, whose concentration in plasma is in a inverse correlation with incidence of coronary heart disease.

This thesis examines the effect of remodelling HDL on the metabolism of apo A-I. The major focus is on the effects of CETP and LCAT in the regulation of apo A-I concentration in HDL. Chapter one reviews the literature with respect to the intraplasma metabolism of lipoproteins and the remodelling of HDL by various plasma factors. Chapter two describes the general methods and materials used in the studies presented in this thesis.

In chapter three the effects of incubation of HDL with CETP in the presence of VLDL and/or LDL on apo A-I concentration in HDL are examined. It is demonstrated that coincident with the reduction in HDL particle size, a proportion of apo A-I dissociated from HDL during the incubation. The dissociation of apo A-I was time-dependent. The percentage of apo A-I that dissociated from HDL correlated positively with the

concentrations of VLDL, LDL, and CETP but negatively with the concentration of HDL in the incubation.

The characterization of the dissociated apo A-I from HDL is presented in Chapter four. The dissociated apo A-I was essentially free of cholesterol and phospholipid and was not associated with other apolipoproteins. When subjected to agarose gel electrophoresis, the dissociated apo A-I migrated to a prebeta position which is identical to that of purified, lipid-free apo A-I. This is distinct from the alpha migration of the bulk HDL in plasma.

Studies in chapter five demonstrate that the dissociation of apo A-I from HDL mediated by CETP is preventable and reversible in a process dependent on LCAT activity. After the first incubation with CETP, HDL particles reduced in size and lost a proportion of apo A-I. The resulting HDL was isolated from the incubation and reincubated with lipid-free apo A-I in the presence of LCAT. Due to the generation of cholesteryl esters by LCAT, HDL particles became bigger in size. Coincided with this increase in HDL size was a proportional rise in HDL apo A-I content, which was comparable to that of native HDL prior to incubation. The reincorporation of apo A-I into HDL implies a way in which apo A-I is preserved in vivo.

Chapter six explores the mechanism by which HDL apo A-I content is increased. The studies were performed by using homogenous reconstituted HDL (rHDL). The increase in cholesteryl ester content of rHDL by LCAT not only resulted in an increase in rHDL particle size, but also resulted in a rise in the numbers of apo A-I molecules in HDL, from two to three per particle. The incubations conducted in the absence of lipid-free apo A-I did not cause changes in the concentration of rHDL-associated apo A-I, indicating that the increase from two to three molecules of apo A-I per particle was achieved at the expense of a one third reduction in the number of rHDL particles, a process that must have involved particle fusion between HDL. In contrast, when the incubation mixture was supplemented by lipid-free apo A-I, there was an increase in the

concentration of HDL-associated apo A-I of approximately 50%, indicating that under these conditions, the increase from two to three in the number of apo A-I molecules per rHDL particles was achieved by a direct incorporation of lipid-free apolipoproteins without fusion of HDL particles.

In conclusion, these observations provide in vitro evidence of a cyclic dissociation and reassociation of apo A-I with HDL. These processes are regulated by plasma factors which modify HDL lipids. This thesis expands our understanding of the HDL remodelling and the regulation of apo A-I concentration in plasma.