Suppression of Hepatic HMG-CoA Reductase Activity by β-Muricholic Acid in Mice Fed a Diet Containing Cholesterol and Cholic Acid

Yukio FUJINO, Koji NAKAYAMA, Kuniyoshi YOSHIMURA, Yutaka FURUYA and Takara YONAGA
Department of Pharmacology, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173, Japan
Accepted January 30, 1988

Abstract—A diet containing cholesterol and cholic acid (SID) is known to induce the formation of cholesterol fatty liver as well as cholesterol gallstones. The activity of HMG-CoA reductase, one of the key enzymes for cholesterol synthesis in the liver, is significantly lowered by addition of β-muricholic acid to SID. The prevention of fatty liver formation by β-muricholic acid was accompanied by the suppression of HMG-CoA reductase activity.

Addition of cholesterol and cholic acid (CA) to a diet (SID) is known to induce not only cholesterol gallstones (1) but also cholesterol fatty liver in mice (2, 3). A preliminary study reported that a number of bile acids prevented both formations of the cholesterol gallstones and the fatty liver (4). Among the bile acids, β-muricholic acid (MCA), a murine specific bile acid, is most effective for the prevention; this data will be published elsewhere in detail. It is known that hepatic cholesterol synthesis is regulated by "bile salt-cholesterol feedback" (5) as well as the end-product inhibition, "cholesterol-cholesterol feedback" (6, 7). It is also reported that the "bile salt-cholesterol feedback" results from suppression of the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase, one of the key enzymes for cholesterol synthesis in the liver, by bile salts (8). In the present study, in order to clarify the roles of MCA in the prevention of the fatty liver formation induced by SID, the effect of MCA on HMG-CoA reductase activity was examined.

Six groups, each consisting of 10 mice (male, ICR strain, SPF, 5 week old), were fed a natural diet (powder of a commercial chow, Oriental, Tokyo); a diet containing 1% MCA, 1% cholesterol and 0.5% cholic acid (SID); SID plus 1% MCA; SID plus 1% ursodeoxycholic acid (UDCA); and SID plus 1% chenodeoxycholic acid (CDCA) for 3 days. The animals were killed by decapitation. The liver was perfused from the portal vein with ice cold homogenizing medium consisting of 40 mM phosphate buffer, pH 7.2, 30 mM EDTA, 100 mM sucrose, 50 mM KCl and 1 mM dithiothreitol. The liver was quickly removed and minced in the homogenizing medium. After homogenization in an ice-cold glass homogenizer with a Teflon pestle, the microsome fraction was obtained by the procedure described by Ness et al. (9). Cholesterol content of the microsome fraction was analyzed using a commercial kit (Determiner TC-555, Kyowa Medex, Co., Ltd., Tokyo). HMG-CoA reductase activity was measured by the method described by Siedel (10). Protein concentration was determined according to the method of Lowry et al. (11), using bovine serum albumin as the standard.

As indicated in Fig. 1a and b, liver weight was significantly increased and the protein content in the liver decreased by SID feeding regardless of adding bile acids. Single addition of MCA to the diet did not exert any effects. Protein contents in the microsome fraction obtained from mice fed SID and SID plus 1% CDCA increased significantly (Fig. 1c). In the previous study, it was found that
these groups formed the fatty liver when maintained for 14 days with these diets (4). The coincidence of the fatty liver formation and the increase in microsomal protein suggests that microsomes contribute to fatty liver formation.

Cholesterol content in the microsomal fraction significantly increased in all groups receiving SID (Fig. 2a). Enzymes contributing to cholesterol synthesis are known to be localized in the microsomal fraction (12). Cholesterol in the microsomal fraction originates both from endogeneous synthesis and exogeneous transport from the diet. The origin of increased cholesterol in these groups could not be specified in the present study. It will be necessary to study these origins in the future using a labeled compound. Microsomal cholesterol contents per liver weight, calculated from the data indicated in Figs. 1c and 2a, increased in the group maintained with SID and SID plus CDCA. The coincidence of the increase in microsomal cholesterol contents and the fatty liver formation also suggests the contribution of microsomes to the fatty liver formation.

To evaluate the capacity for cholesterol synthesis in these groups, HMG-CoA reductase activity was measured in the microsomal fraction. As shown in Fig. 2c, appreciable HMG-CoA reductase activity was present in the microsomal fraction obtained from the control group. The level of the activity was almost similar to those reported by Siedel (10). Single addition of MCA to the diet did not diminish the activity. The activity substantially decreased in the SID given group. This may indicate the occurrence of both "cholesterol-cholesterol feedback" and "bile salt-cholesterol feedback". The decrease in the activity was more extensive in the groups fed SID containing MCA or UDCA than in the groups fed SID and SID plus CDCA. In the group maintained with SID plus MCA, the activity was undetectably low. In this group, cholesterol
synthesis in liver is assumed to be almost completely blocked. On the contrary, cholesterol synthesis probably occurs in the groups fed SID and SID plus CDCA, though the rates may be considerably lower when compared with the control group. In the preliminary study, CDCA has only a weak effect on the prevention of the cholesterol fatty liver formation induced by SID (4). The coincidence of the extent of the effect on prevention of cholesterol fatty liver formation and the suppression of HMG-CoA reductase activity of bile acids suggests that the protective effect of MCA is, at least in part, due to the decreased cholesterol synthesis in the liver.

The mechanism of inhibition of HMG-CoA reductase activity by MCA is not known at present. In a preliminary experiment using crude HMG-CoA reductase, tauro-β-muricholic acid did not inhibit significantly the enzyme activity. Therefore, the inhibitory action of MCA, which becomes tauro-β-muricholic acid in mouse liver, is probably due to the indirect inhibition of this enzyme.

References