DIETARY SILYMARIN IMPROVES REMOVAL OF LOW DENSITY LIPOPROTEINS BY THE PERFUSED RAT LIVER

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Silymarin administered to the rats concurrently with high cholesterol diet normalized the high cholesterol diet-induced retardation of disapperance of low density lipoprotens (LDL) from the medium during recirculating perfusion of livers from these rats. We suggest that the improvement of LDL removal by the liver after silymarin treatment contributes to the antihypercholesterolemic effect of silymarin.

INTRODUCTION

Previously we have shown that peroraly administered silymarin, a mixture of antioxidant flavonolignans from medicinal plant *Silybum marianum* (L.), prevents both the accumulation of exogenous cholesterol in the liver and the development of diet-induced hypercholesterolemia in rat[1, 2]. The silymarin-induced decrease of plasma cholesterol has been associated with a decrease in apo B containing lipoproteins VLDL and LDL, and an increase in HDL[1, 2], changes considered to be of benefit in pharmacological treatment of hypercholesterolemia.

The removal of LDL by the liver represents one from the most important mechanisms regulating the level of plasma LDL[3]. To investigate if silymarin treatment can improve the removal of LDL by the liver, we used a model of liver perfusion. The liver of rat fed high cholesterol diet with or withouth silymarin was perfused with LDL and the disappearance of LDL from perfusion medium was determined.

MATERIAL AND METHODS

Female Wistar rats (160–170 g) kept in standard laboratory conditions with free access to water and diet were randomly ascribed into 3 groups fed 1) standard diet (STD), 2) high cholesterol diet (HCD) prepared by adding 1 % (w/w) of cholesterol and 10 % (w/w) of lard fat to standard diet, and 3) high cholesterol diet supplemented with 1 % (w/w) of silymarin (HCD+ 1 % SM). Silymarin was a kind gift from Dr. L. Cvak (Galena Opava, Czech Republic). All groups of rats were maintained on the diets for 19 days, and fasted overnight before liver perfusion. The rats were anesthetized with Hypnorm (0.05 ml/100 g b.w., Janseen, Belgium) and Stesolid (0.05 ml/100 g b.w., Kabi-Pharmacia, Sweden). The livers were perfused as described previously[4]. After 10 min washing of blood in a single pass mode, the livers were perfused for 30 min with recirculating medium. This medium contained LDL (1.94 mg of cholesterol per liver) isolated by sequential ultracentrifugation[5] in 50.4 Ti Beckman rotor from human plasma containing Na_2EDTA (1 mg/ml), Aprotinin (10 U/ml, Sigma, USA) and NaN_3 (0.1 mg/ml). Samples of 0.04 ml were taken from the perfusion medium at the times indicated in Fig. 1. Cholesterol was assayed by enzymatic method (Bio-La-Test Oxochrom Cholesterol 250 E, Lachema, Czech Republic).

RESULTS

Fig. 1 shows the disappearances of LDL cholesterol from recirculating medium during perfusion of livers from rats fed standard diet, high cholesterol diet or high cholesterol diet supplemented with silymarin. Feeding of rats with high cholesterol diet significantly slowed the liver removal of LDL cholesterol when compared to the liver of rat fed standard diet. When the high cholesterol diet was supplemented with silymarin, the removal of LDL cholesterol by the liver was normalized, since the disappearance reached values observed in the liver of rats fed standard diet.

DISCUSSION

Silymarin administered concurrently with high cholesterol diet caused acceleration of LDL removal by the perfused rat liver. In our previous experiments we have shown, that supplementation of high cholesterol diet with silymarin is associated with the decrease in liver cholesterol[2] . This is probably the main base of silymarin-induced improvement of removal of LDL by the liver, since is known that the liver LDL uptake is regulated by the LDL-receptor activity, which increases with the fall of cholesterol content in the liver[3].

However, in the dose-dependent study we have shown that the silymarin-induced decrease in plasma cholesterol level precedes the decrease in liver cholesterol content[2]. This suggested that silymarin could act, in addition, directly at the membrane of hepatocyte. It is known that silyma-

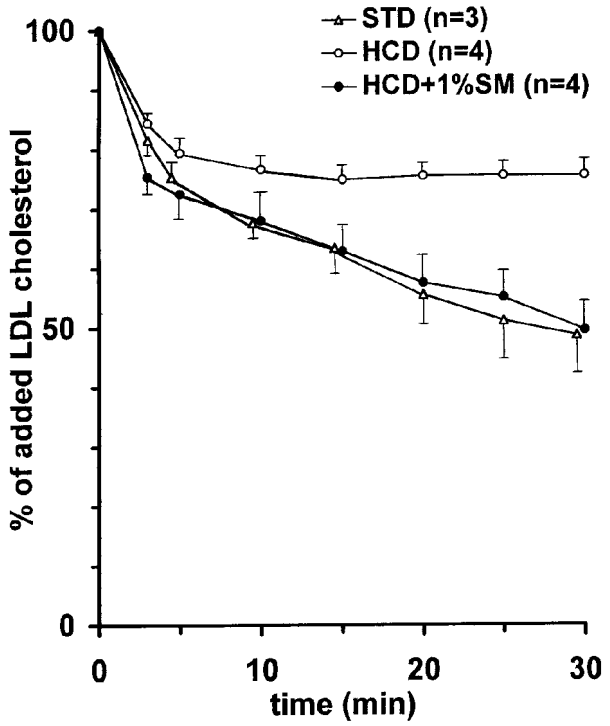

Fig. 1. Disappearance of LDL from medium during perfusion of livers of rats fed standard diet (STD), high cholesterol diet (HCD) and high cholesterol diet supplemented with silymarin (HCD +1% SM). Data points are means ± SE.

rin exerts antioxidant and membrane stabilizing activities[6], attributes important for liver secretion and uptake of plasma lipoproteins. In paracetamol-damaged rat hepatocytes silymarin was found to normalize the decreased binding of LDL[7].

In conclusion, our study has demonstrated that supplementation of high cholesterol diet with silymarin improves the liver LDL removal contributing to the antihypercholesterolemic effect of silymarin.

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