Probucol Decreases Mevalonate Pyrophosphate Decarboxylase in the Rat Liver

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It is known that cholesterol biosynthesis in the liver is inhibited by probucol. This inhibition by probucol is caused at least in part by a decrease in 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity. In this study, we examined serum cholesterol and the change in the activity or protein level of mevalonate pyrophosphate decarboxylase (MPD), which is involved in cholesterol biosynthesis, in the livers of rats fed probucol. The results indicated that serum cholesterol, MPD activity and MPD protein were decreased by 70, 50 and 60% by probucol, respectively, as compared with those in rats fed normal chow. These data show for the first time that probucol decreases the level of an enzyme involved in cholesterol biosynthesis other than HMG-CoA reductase.

Key words mevalonate pyrophosphate decarboxylase; probucol; cholestyramine; rat; liver

In the first steps of the biosynthesis of cholesterol from acetic acid, mevalonate pyrophosphate decarboxylase (MPD) catalyzes a bimolecular reaction between mevalonate pyrophosphate and ATP to form isopentenyl pyrophosphate,1—3) inorganic phosphate, adenosine 5'-diphosphate (ADP), and CO₂. Although 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase is the rate-limiting enzyme of this pathway, MPD is also considered to be a regulatory enzyme based on the effects of dietary cholesterol or cholesterol-lowering drugs.4—7)

Probucol is a cholesterol-lowering drug. The main action of probucol in reducing serum cholesterol is an effect the uptake of low density lipoprotein (LDL), not the LDL-mediated receptor, by increasing the catabolic excretion of cholesterol into bile, although probucol inhibits the incorporation of acetate into cholesterol and the intestinal adsorption of cholesterol. In rats, HMG-CoA reductase activity was unchanged during the first 2 weeks of probucol treatment, and more prolonged probucol (4 weeks) led to inhibition of the activity (70%) of this enzyme and a reduction of sterol synthesis (60%), although the decrease of plasma cholesterol in rats given probucol for 4 weeks (50%) was unchanged compared to that in rats given probucol for 2 weeks (45%).8) These data suggested that the inhibition of cholesterol synthesis was not directly responsible for the initial reduction in plasma cholesterol. In mice, decreases of serum cholesterol (45—65, 60%), incorporation of [14C]-acetate into cholesterol (45, 60—70%) and HMG-CoA reductase (30%, no data) were caused by treatment with probucol at 400 or 800 mg/kg for 7 d, respectively.9) These data suggested that the inhibition of cholesterol synthesis was responsible for the reduction in serum cholesterol. Also, when [14C]-acetate or [14C]-mevalonate was injected intravenously in mice given probucol at 400 mg/kg for 7 d, the reduction of incorporation into cholesterol in serum was 23 or 11%, respectively, as compared with that in nontreated mouse. These data suggested the possibility that probucol inhibited some enzyme involved in cholesterol biosynthesis other than HMG-CoA reductase.

In this study, we examined the change in the activity or protein level of MPD, which is involved in cholesterol biosynthesis, by probucol. The results showed that MPD in the rat liver was decreased by probucol.

MATERIALS AND METHODS

Materials Probucol was kindly provided by Daiichi (Tokyo); Cholestyramine was from Bristol Laboratories; and Cholesterol CII-test Wako was from Wako (Tokyo). All other chemicals were of reagent grade and purchased from commercial sources.

Animals Male Wistar Kyoto rats (10 weeks old, 280 g) were housed in a light-controlled room (light phase, 6:00—18:00). They were fed powdered chow with or without 0.5% probucol or 5% cholestyramine. The rats were fed 10 g of the chow a day on average.

Preparation of Crude Extract A crude extract of liver was prepared according to the method of Michihara et al.10) Immunoblot Procedures Immunoblot analysis was carried out as described by Michihara et al.10) The signals were measured with a Shimadzu Chromatoscanner (S-910) (Shimadzu, Tokyo).

Radioactive Assay Enzyme activities in the crude extract were measured according to the method of Sawamura et al.11)

Protein Determination Proteins were determined by the method of Lowry et al.,12) with bovine serum albumin as the standard.

Serum Cholesterol Serum cholesterol was determined by means of the cholesterol CII-test Wako.

RESULTS

Serum Cholesterol in Rats Fed Probucol or Cholestyramine To verify that probucol decreases serum cholesterol, the serum cholesterol in rats fed probucol was measured using a cholesterol CII-test Wako. As shown in Fig. 1, serum cholesterol was decreased by 65, 70, and 68% by the administration of probucol for 5, 10, and 15 d, respectively, as compared with the levels in rats fed normal chow for the same period. These data indicate that serum cholesterol was significantly decreased by the administration of probucol for 5 d. Also, these data were similar to the reported data of mice fed probucol for 7 d (45—65%), but different from those of rats fed probucol for 2 weeks (45%).

Cholestyramine is a cholesterol-lowering drugs that causes
inhibition of the intestinal adsorption of cholesterol and increased catabolic excretion of cholesterol into the bile. The effect of cholestyramine is similar in part to the effect of probucol. Therefore, we also examined the change in the activity or protein level of MPD and the level of serum cholesterol in response to treatment with cholestyramine, to clarify the differences of the effects between probucol and cholestyramine. As shown in Fig. 1, serum cholesterol was decreased by 37, 40, and 35% in rats fed cholestyramine for 5, 10, and 15 d, respectively, as compared with the levels in rats fed normal chow for the same period. These data indicate that the serum cholesterol was lower rats treated with probucol than in rats treated with cholestyramine.

**Immunoblot Analysis of MPD in Rats Treated with Probucol or Cholestyramine**

The results of immunoblot analysis performed with an anti-MPD antibody using the livers of rats fed probucol, cholestyramine or normal chow are shown in Fig. 2. The amount of MPD was decreased by 60, 70, and 50% by probucol administration for 5, 10, and 15 d, respectively, as compared with the corresponding values in rats fed normal chow for the same period. These data showed for the first time that probucol decreases an enzyme involved in cholesterol biosynthesis other than HMG-CoA reductase. Also, the amount of MPD was increased 2.7, 2.5, and 2.8-fold by administration of cholestyramine for 5, 10, and 15 d, respectively, as compared with the corresponding values in rats fed normal chow for the indicated period.

**MPD Activity in Livers of Rats Treated with Probucol or Cholestyramine**

MPD activity in the livers of rats fed probucol, cholestyramine or normal chow, is shown in Fig. 3. MPD activity was decreased by 50, 55, and 50% by administration of probucol for 5, 10, and 15 d, respectively, as compared with the corresponding values in rats fed normal chow for the same period. Also, MPD activity was increased 2.0, 2.5, and 2.2-fold by administration of cholestyramine for 5, 10, and 15 d, respectively, as compared with the corresponding values in rats fed normal chow for the same period.

These data indicate that the effects of probucol and cholestyramine on the MPD activity in the livers of rats were approximately proportional to the effects on the protein level.

**DISCUSSION**

The serum cholesterol level in rats treated with probucol was significantly lower than that in rats treated with cholestyramine. Also, the level of MPD was increased by cholestyramine, but decreased by probucol (Fig. 4). From
these data, we considered it possible that the difference in the decrease of serum cholesterol between probucol and cholestyramine was the result of differences in the inhibition of cholesterol biosynthesis caused by decreases of MPD and HMG-CoA reductase. Probucol may further decrease serum cholesterol by increasing in the uptake of LDL, in addition reducing cholesterol in the liver by not only increasing the catabolic excretion of cholesterol into bile but also inhibiting cholesterol biosynthesis. Thus, although the main effect of probucol in reducing serum cholesterol was on the uptake of LDL, another effect of probucol may have been the inhibition of cholesterol biosynthesis.

Our results were similar to the results previously reported in mice, as described in the Introduction, but different from the results reported in rats. From the data reported in mice, it was concluded that the inhibition of sterol synthesis was increased as the dose of probucol increased. The doses of probucol used in this study were similar to those used in mice (400 mg/kg), whereas the previously reported dose used in rats was 1/10 that used in mice. This suggests that the reductions of sterol synthesis, HMG-CoA reductase and MPD activity were caused by different doses of probucol. From our data and the previously reported data, we concluded that the decrease of cholesterol synthesis by a high concentration of probucol was involved in the decrease of serum cholesterol, but that caused by a low concentration of probucol was not.

In conclusion, we showed that probucol decreased the level of MPD, an enzyme involved in cholesterol biosynthesis. Further studies will be necessary to understand the mechanism by which probucol reduces the level of MPD.

REFERENCES