Influence of probucol on cholesterol and lipoprotein metabolism in man

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Abstract The mechanisms for the hypocholesterolemic action of probucol were examined in 17 patients with various levels of plasma cholesterol and triglycerides (TG). All the patients were studied on a metabolic ward. The first period of 6 weeks was for control. Thereafter, probucol was started, and after 2–6 months of drug treatment, the patients were readmitted for another 6-week period for a repeat study. During treatment with probucol, the cholesterol decreased in total plasma by an average of 12%, in low density lipoproteins (LDL) by 11%, and in high density lipoproteins (HDL) by 9%. The TG in total plasma and in very low density lipoproteins (VLDL) remained unchanged during probucol treatment. Turnover of low density lipoprotein apoprotein (apoLDL) was estimated following injection of ^{125}I-labeled apoLDL. Probucol increased the fractional catabolic rate (FCR) for apoLDL by an average of 23%, but did not change apoLDL synthesis. The drug produced no consistent changes in fecal excretions of cholesterol (neutral steroids) and bile acids, in cholesterol absorption, in lipid composition of gallbladder bile, in biliary secretion of cholesterol and bile acids, or in the activities of lipoprotein lipase and hepatic lipase. These data show that probucol lowers LDL by increasing its catabolism. This effect appears to be independent of any changes in metabolism of cholesterol or bile acids.—**Kesäniemi, Y. A., and S. M. Grundy.** Influence of probucol on cholesterol and lipoprotein metabolism in man. *J. Lipid Res.* 1984. **25:** 780–790.

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Probucol is a plasma cholesterol-lowering drug without structural similarities to other available lipid-lowering agents (1). It reduces plasma total cholesterol approximately 10–20% by lowering levels of both low density lipoproteins (LDL) and high density lipoproteins (HDL) (2–15). Plasma triglycerides (TG) and very low density lipoproteins (VLDL) levels usually remain unchanged, but may be reduced in some hypertriglyceridemic patients (8), and increased in some patients with familial hypercholesterolemia (6).

The mechanisms by which probucol reduce plasma LDL and HDL are unknown. The drug has been reported to increase fecal bile acids transiently (6, 16) and possibly inhibit cholesterol synthesis (6). In vitro measurements of cholesterol synthesis in intestine, however, have not confirmed the latter effect (17–19). Other suggested actions are decreased cholesterol absorption and reduced lipoprotein formation (6, 17, 18, 20).

Although the degree of LDL-lowering by probucol is not marked, several observations suggest that the drug may be a useful adjunct in treatment of hypercholesterolemia. Probucol is easy to take and has few side effects (1, 21). It potentiates LDL-lowering of bile acid sequestrants (22). It has been reported to cause regression of xanthomata and xanthelasma (23, 24). Finally, despite lowering of HDL, probucol-treated subjects have been reported to have a lower 5-year incidence of sudden death and myocardial infarction in a primary prevention trial (25).

This study was designed to examine the effects of probucol on cholesterol and lipoprotein metabolism in man. Specifically, the following questions were addressed. Does probucol lower LDL by decreasing production or by increasing clearance of this lipoprotein? What are the effects of probucol on synthesis and excretion of cholesterol and bile acids immediately after starting the medication and later in a steady state? Do changes in sterol balance explain the reduction in plasma cholesterol and lipoproteins? Does probucol influence intestinal absorption of cholesterol or metabolism of biliary lipids?

METHODS

Patients

Seventeen patients were studied on the Special Diagnostic and Treatment Unit of the Veterans Administration Medical Center, San Diego, CA. Sex, age, body habi-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triglyceride; FCR, fractional catabolic rate; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; FA, fatty acid; U/P, urine/plasma ratio of ^{125}I.

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tus at time of admission, and diagnosis of each patient are presented in **Table 1.** At first admission, plasma cholesterol of the patients ranged from 162 to 322 mg/dl with a mean of 263 mg/dl (**Table 2**). Plasma TG ranged from 130 to 583 mg/dl with a mean of 245 mg/dl, and VLDL-TG ranged from 57 to 504 mg/dl with a mean of 186 mg/dl. LDL-cholesterol varied between 104 and 238 mg/dl with a mean value of 162 mg/dl and HDL-cholesterol varied between 27 and 70 mg/dl with a mean of 44 mg/dl. One patient (#17) had mild hyperglycemia treated with sulfonylurea; all others had normal fasting and 2-hr postprandial glucose. Several had clinical atherosclerotic disease, but none had congestive heart failure, nor evidence of liver or gastrointestinal disease. None had previous cholecystectomy. All patients gave informed consent for the investigation, which was approved by the Human Studies Committee of the University of California, San Diego.

Experimental design

Patients were studied during two periods, each of approximately 6 weeks duration. The first period was for control and a placebo was given. Thereafter all except five patients were discharged, and probucol (500 mg twice daily) was started. After probucol therapy for 2–6 months, the patients were readmitted to the metabolic unit for a second 6-week period. Five patients (#9, 14–17) remained in the hospital during initial probucol therapy to determine immediate effects of the drug. All the patients received a diet of solid food and liquid formula, which was identical each day, throughout both periods. Plasma lipids and lipoproteins were estimated twice weekly. An additional series of metabolic studies was carried out on as many patients as possible. For various reasons some patients were unable to undergo all studies. The particular patients receiving each test are indicated in the tables.

The general design of the study was as follows. In most patients cholesterol balance measurements were carried out throughout both 6-week periods. In addition, cholesterol balance was determined immediately after starting probucol treatment in four patients (#9, 14, 16, 17). Intestinal cholesterol absorption was measured during the second or third week of each period. Low density lipoprotein apoprotein (apoLDL) turnover was carried out during the last 3 weeks of each period. Postheparin lipolytic enzymes were measured during the third or fourth week of each period. Lipid composition of gallbladder bile was determined during the second and third week of each period. Finally, at the end of each period, estimations of hepatic secretions of biliary lipids and of pool sizes of bile acids were made.

All patients tolerated probucol well. One patient (#8) had diarrhea and abdominal cramps as a side effect of the drug. However, he was able to continue the medication, and he completed the study. No side effects were observed in the other patients.

Diets

The metabolic diet consisted of mixed solid food and liquid formula containing 40% of calories as fat, mostly in the form of lard. The basic composition and pattern of this diet have been described previously in detail (26).

TABLE 1. Clinical data

Patient	Age	Sex	Ideal Weight	Weight: Control Period	Weight: Probucol Period	Clinical Diagnosis[a]
	yrs	*F/M*	*%*	*kg*		
1	58	M	114	67	67	Normal
2	44	M	132	73	73	Normal
3	63	M	114	89	88	Normal
4	31	M	100	72	72	Normal
5	62	M	114	75	72	Normal
6	61	M	122	87	88	Normal
7	60	M	82	60	57	Normal
8	57	M	113	74	76	CHD
9	59	F	127	75	78	Normal
10	66	M	118	79	80	CVD
11	62	M	106	83	84	CHD
12	58	M	185	113	111	PVD
13	59	M	114	80	81	CHD
14	62	M	118	92	91	Normal
15	59	M	99	73	72	CVD
16	60	F	128	69	68	Normal
17	55	M	156	100	100	CVD, MH

[a] CHD, Coronary heart disease; CVD, cerebrovascular disease; PVD, peripheral vascular disease; MH, mild hyperglycemia.

TABLE 2. Plasma lipids and lipoproteins during control and probucol-treatment periods

Patient	Period[a]	Total Chol	Total TG	VLDL-TG	LDL-Chol	HDL-Chol
			mg/dl ± SEM			
1	I	230 ± 7 (9)[b]	188 ± 12	133 ± 11	149 ± 4	47 ± 2
	II	215 ± 4 (9)	213 ± 18	153 ± 18	137 ± 7	37 ± 2[c]
2	I	317 ± 8 (9)	583 ± 45	504 ± 41	141 ± 4	35 ± 2
	II	242 ± 5 (7)[c]	530 ± 75	439 ± 67	128 ± 12	24 ± 1[c]
3	I	285 ± 5 (10)	210 ± 11	152 ± 10	192 ± 5	52 ± 1
	II	285 ± 6 (10)	209 ± 9	147 ± 9	195 ± 4	51 ± 2
4	I	260 ± 6 (9)	241 ± 11	175 ± 9	177 ± 7	34 ± 3
	II	219 ± 6 (9)[c]	340 ± 32[d]	257 ± 31[d]	113 ± 7[c]	27 ± 1[c]
5	I	201 ± 3 (7)	109 ± 6	65 ± 5	130 ± 4	56 ± 1
	II	187 ± 4 (9)[c]	90 ± 6[c]	51 ± 4[c]	120 ± 4	54 ± 1
6	I	239 ± 3 (9)	149 ± 5	99 ± 5	170 ± 3	45 ± 1
	II	191 ± 8 (9)[c]	154 ± 5	104 ± 5	134 ± 5[c]	31 ± 3[c]
7	I	267 ± 5 (13)	193 ± 7	136 ± 7	194 ± 4	38 ± 1
	II	228 ± 7 (8)[c]	190 ± 14	132 ± 12	159 ± 6[c]	34 ± 1[c]
8	I	322 ± 5 (10)	167 ± 5	115 ± 5	231 ± 5	61 ± 2
	II	270 ± 7 (8)[c]	130 ± 6[c]	81 ± 5[c]	202 ± 5[c]	48 ± 2[c]
9	I	270 ± 4 (10)	116 ± 8	57 ± 6	184 ± 4	70 ± 1
	II	247 ± 4 (9)[c]	112 ± 10	53 ± 7	172 ± 2[c]	61 ± 1[c]
10	I	251 ± 9 (4)	300 ± 11	242 ± 9	146 ± 9	27 ± 2
	II	206 ± 3 (14)[c]	234 ± 9[c]	180 ± 10[c]	133 ± 3	31 ± 1
11	I	239 ± 3 (7)	340 ± 46	276 ± 48	108 ± 6	28 ± 2
	II	206 ± 2 (9)[c]	286 ± 7	230 ± 6	123 ± 2[d]	35 ± 1[d]
12	I	315 ± 27 (9)	405 ± 48	340 ± 44	128 ± 4	30 ± 0.3
	II	298 ± 11 (6)	375 ± 17	302 ± 20	113 ± 8	29 ± 4
13	I	204 ± 7 (10)	130 ± 7	83 ± 6	138 ± 4	46 ± 2
	II	180 ± 2 (9)[c]	81 ± 2[c]	43 ± 2[c]	119 ± 3[c]	48 ± 2
14	I	281 ± 8 (11)	193 ± 9	137 ± 8	194 ± 6	49 ± 2
	II	263 ± 5 (8)	148 ± 8[c]	98 ± 7[c]	190 ± 4	48 ± 1
15	I	162 ± 3 (8)	149 ± 8	91 ± 9	104 ± 10	45 ± 4
	II	163 ± 4 (9)	201 ± 16[d]	152 ± 14[d]	78 ± 4[c]	48 ± 1
16	I	322 ± 4 (5)	548 ± 77	470 ± 70	135 ± 7	29 ± 2
	II	283 ± 6 (8)[c]	519 ± 19	442 ± 21	136 ± 11	29 ± 2
17	I	309 ± 6 (6)	136 ± 10	82 ± 9	238 ± 7	50 ± 1
	II	268 ± 5 (7)[c]	115 ± 5	69 ± 4	199 ± 5[c]	52 ± 1
Mean ± SEM	I	263 ± 11 (17)	245 ± 35	186 ± 33	162 ± 10	44 ± 3
	II	232 ± 10 (17)[e]	231 ± 33	172 ± 30	144 ± 9[e]	40 ± 3[e]

[a] Period I, control; Period II, probucol.
[b] Number of determinations in parentheses.
[c] Period II significantly lower than Period I by Student's *t*-test ($P < 0.05$ or less).
[d] Period II significantly higher than Period I by Student's *t*-test ($P < 0.05$ or less).
[e] Period II significantly lower than Period I by paired *t*-test ($P < 0.05$ or less).

The patients were given three liquid meals and one solid-food meal per day. Calories were divided approximately equally between the feedings. Liquid formulas were given at 8:30 AM, 1:00 PM, and 7:00 PM and contained 15% of calories as milk protein, 45% as dextrose, and 40% as fat (lard). These diets were prepared by Hospital Diet Products, Organon Corp., Buena Park, CA (courtesy of Mr. Fred Teixeira). One solid-food meal was given at 11:00 AM, and it contained dry cereal (corn flakes), nonfat bread, skim milk, added fat (as lard), and sugar for coffee. Fat comprised approximately 40% of calories in solid-food meals. Cholesterol intakes varied between 84 and 150 mg/day. Vitamin and mineral supplements were given daily. Each patient was weighed daily, and caloric

intake was adjusted to maintain total body weight at a constant level throughout the control and probucol periods.

Cholesterol and bile acid metabolism

Cholesterol balance studies were carried out on 16 patients as described previously (27–30). Percentage absorption of cholesterol was measured on ten patients by the fecal isotope ratio of Crouse and Grundy (31). On eight patients, the lipid composition of fasting gallbladder bile was obtained during each of the two study periods as described previously (26); for this measurement bile was obtained by duodenal intubation and stimulation of gallbladder contraction. In four patients, hourly outputs of biliary cholesterol, bile acids, and phospholipids were determined during constant duodenal infusion of liquid formula (32). The pool size of bile acids was measured simultaneously (26).

Plasma lipids and lipoproteins

Blood for plasma lipid and lipoprotein quantification was obtained twice weekly after a 12-hr fast. Total cholesterol and TG were determined on a Technicon AutoAnalyzer (Model II, Technicon Instruments Corp., Tarrytown, NY) (33, 34). Concentrations of cholesterol and triglycerides in VLDL, LDL, and HDL were estimated as described in the Lipid Research Clinics Manual of Laboratory Operations (35).

Low density lipoprotein turnover studies

Turnover rates of apoLDL were measured on nine subjects as described previously (36). After the patients had been on mixed solid food and liquid formula for 10 days to 2 weeks, plasmapheresis was carried out for lipoprotein isolation. LDL (d 1.025–1.060 g/ml) was isolated, and the protein moiety was labeled with ^{125}I by the iodine-monochloride method of McFarlane (37) as modified by Bilheimer, Eisenberg, and Levy (38). The labeled LDL was injected intravenously; it contained 50–70 μCi of radioactivity and 2–10 mg of protein. Blood samples were collected at 5, 10, 15, 20, 30, and 60 min, 3, 6, 9, 12, 24, 36, and 48 hr, and daily (fasting) thereafter for 14–21 days. Determinations of radioactivity and concentrations of total cholesterol and triglyceride were made on each plasma sample. Lipoprotein lipid and protein quantification was done biweekly throughout the study.

Urine specimens were collected in bottles containing an alkaline preservative (39). The patients received 0.5–0.9 gm of KI orally in divided doses each day to suppress ^{125}I uptake by the thyroid. The fractional catabolic rate (FCR) and synthesis rate for apoLDL were determined as previously described (36). Briefly, two exponential components of the die-away curve of plasma radioactivity were fit with the two-pool Matthews model (40); the data were used to calculate an FCR for plasma apoLDL. The FCR was measured independently by relating the daily urinary excretion rate of ^{125}I radioactivity to the ^{125}I radioactivity in plasma (U/P ratio) as described previously (36). The concentration of apoLDL was calculated from the mean of the four to six measured values for LDL-cholesterol and the four to six measured ratios of protein-to-cholesterol in each patients' LDL. These measurements were done biweekly throughout the study. LDL protein was determined by a modified method of Lowry et al. (41) as described by Sata, Havel, and Jones (42).

Plasma postheparin lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL)

Postheparin plasma was obtained from blood drawn 15 min after intravenous injection of 60 IU/kg sodium heparin (Riker Labs., Inc.). Subjects had been fasting for 14 hr prior to sampling. Samples were cooled immediately on ice and centrifuged at 4°C for 30 min at 480 *g*. The plasma was removed and recentrifuged for 30 min at 750 *g* at 4°C. Samples were then stored at −70°C up to 3 months before assay. LPL and HTGL activities were determined as described by Baginsky and Brown (43).

RESULTS

Plasma lipids and lipoproteins

Values for concentrations of plasma cholesterol, TG and VLDL-TG, LDL, and HDL cholesterol in each subject during control and probucol treatment periods are shown in Table 2. Overall, probucol lowered plasma total cholesterol 12% and LDL cholesterol 11%; at the same time, a 9% decrease was observed in HDL cholesterol. Probucol produced no overall change in plasma total or VLDL-TG. Mean values for plasma postheparin lipoprotein lipase activities in twelve patients were unchanged between control (11.4 μmol of FA/hr per ml ± 1.0 (SE)) and probucol (12.3 μmol of FA/hr per ml ± 1.3) periods. Also, plasma postheparin hepatic triglyceride lipase activities were unaffected by probucol treatment (control, 32.2 ± 3.9 vs. probucol, 31.7 ± 4.5 μmol of FA/ml per hr; n = 12).

ApoLDL kinetics

Table 3 shows kinetic parameters for ^{125}I-labeled apoLDL in nine patients during control and probucol-treatment periods. Probucol lowered plasma apoLDL concentration in seven subjects and increased it in two. Plasma apoLDL was decreased by an average of 10.5% by drug treatment. The mean ratio of protein-to-cholesterol in LDL was unaffected by probucol treatment. The FCR for apoLDL as calculated from the die-away curve of plasma radioactivity showed an increase in eight

TABLE 3. Kinetic parameters for ^{125}I-labeled apoLDL turnover during control and probucol-treatment periods

Patient	Period[a]	Plasma Volume	Plasma apoLDL	LDL Prot/Chol	FCR[b] I	FCR[b] II	Rate of Synthesis of apoLDL
		ml	*mg/dl*	*ratio*	*day^{-1}*		*mg/day per kg*
1	I	3148	128	0.86	0.295	0.264	17.9
	II	3041	114	0.83	0.400	0.380	20.7
2	I	3166	106	0.75	0.434	0.347	20.0
	II	2879	118	0.92	0.593	0.511	27.5
3	I	4136	154	0.80	0.330		23.5
	II	3862	165	0.85	0.449	0.401	32.4
4	I	3067	142	0.80	0.441	0.445	26.9
	II	2774	123	1.09	0.560	0.625	26.4
5	I	3671	92	0.71	0.289	0.264	13.0
	II	3391	84	0.70	0.297	0.351	11.7
6	I	3667	124	0.73	0.250	0.289	13.1
	II	3572	117	0.87	0.327	0.317	15.6
7	I	3193	167	0.86	0.298	0.230	26.4
	II	2742	119	0.75	0.333	0.303	19.0
8	I	2835	157	0.68	0.260	0.205	15.6
	II	3132	133	0.66	0.251		13.8
9	I	2820	128	0.70	0.236	0.241	11.4
	II	2650	98	0.57	0.269	0.297	8.9
Mean ± SEM	I (9)	3300 ± 146	133 ± 8	0.77 ± 0.02	0.315 ± 0.025	0.286 ± 0.027[c]	18.6 ± 2.0
	II (9)	3116 ± 138	119 ± 7[d]	0.80 ± 0.05	0.387 ± 0.041[e]	0.398 ± 0.041[c,e]	19.5 ± 2.6

[a] Period I, control, Period II, probucol.
[b] Fraction of intravascular apoLDL pool metabolized each day, calculated either from the plasma die-away curve (I) or from U:P ratio (II).
[c] Only eight determinations.
[d] Period II significantly lower than Period I by paired *t*-test ($P < 0.05$).
[e] Period II significantly higher than Period I by paired *t*-test ($P < 0.005$ or less).

patients and a decrease in one subject; the mean FCR for apoLDL for the whole group was increased 22.9%. Similar results were obtained when FCR was calculated from U/P ratios. All seven patients in whom U/P ratios were determined during both control and probucol-treatment periods showed an increase in FCR with an average value of 34.0%. Some minor changes were noted in the synthetic rates of apoLDL between control and probucol-treatment periods, but, on the average, the drug did not change apoLDL synthesis.

Biliary lipid metabolism

Lipid composition and cholesterol saturation indices of gallbladder bile in eight patients during control and probucol treatment are presented in **Table 4.** Probucol did not change the molar % cholesterol, bile acids, or phospholipids in fasting gallbladder bile. The percentage saturation of gallbladder bile with cholesterol was not increased by probucol. Also, the mean hourly outputs of biliary cholesterol (37 mg/hr ± 8 (SE)), bile acids (755 mg/hr ± 174), and phospholipids (236 mg/hr ± 52), and bile acid pool size (2342 mg ± 139) measured in four patients during the control period were unaffected by probucol treatment (cholesterol, 732 mg/hr ± 121; bile acids, 732 mg/hr ± 121; phospholipids, 266 mg/hr ± 25; bile acid pool size, 2527 mg ± 349). In addition, the mean molar percentages of cholesterol (5.2% ± 0.4), bile acids (79.1% ± 1.8), and phospholipids (15.8% ± 1.4) of these stimulated hepatic bile samples during the control period remained unchanged on probucol treatment (cholesterol, 4.7% ± 0.4; bile acids, 76.7% ± 1.9; phospholipids, 18.6% ± 2.6).

Cholesterol absorption

The percentage absorption of cholesterol during control and probucol-treatment periods were determined on ten patients. Probucol lowered percent absorption in three patients and increased it in three subjects without any overall change among all the ten patients studied (control period, 45% ± 5 (SE) vs. probucol period, 43% ± 5).

Cholesterol balance

Table 5 presents steady-state fecal output of cholesterol, bile acids, and total steroids as well as cholesterol

TABLE 4. Lipid Composition of gallbladder bile during control and probucol-treatment periods

Patient	Period (n)[a]	Cholesterol	Bile Acids	Phospholipids	Bile Saturation (10% solids)
			molar %		%
1	I (1)	7.5	72.9	19.6	111
	II (3)	8.2	74.5	17.3	133
2	I (2)	13.4	64.1	22.5	177
	II (1)	9.9	70.7	19.5	145
3	I (2)	6.5	75.4	18.1	119
	II (1)	7.6	76.0	16.4	129
4	I (1)	10.4	72.8	16.9	168
	II (2)	5.3	78.4	16.3	92
5	I (1)	9.7	71.1	19.3	144
	II (2)	9.7	71.7	18.7	146
6	I (3)	9.2	71.9	18.9	139
	II (2)	9.8	73.0	17.3	157
11	I (2)	7.2	74.0	18.8	117
	II (2)	8.3	71.6	20.1	121
13	I (2)	9.4	70.1	20.5	135
	II (2)	12.0	64.5	23.5	155
Mean ± SEM	I (8)	9.2 ± 0.8	71.5 ± 1.2	19.3 ± 0.6	139 ± 8
	II (8)	8.9 ± 0.7	72.6 ± 1.5	18.6 ± 0.9	135 ± 8

[a] Period I, control; Period II, probucol; n, number of determinations.

balance in 16 patients during control and probucol therapy. The mean fecal excretions of neutal steroids, bile acids, and total steroids were unchanged by the drug, and mean cholesterol balance was similar on control and probucol therapy.

Fecal steroid excretion and cholesterol balance were determined weekly immediately after starting probucol in four patients (**Table 6**). Patient #16 increased fecal outputs of bile acids significantly ($P < 0.05$) from 812 mg/day ± 37 (SEM) to 970 mg/day ± 44 during the first 4 weeks after starting probucol. Variable increases in fecal bile acids were found in patients 9, 14, and 16 during the first week after starting the drug. However, patient #17 showed a decrease in fecal bile acids in the first 3 weeks on probucol even though his plasma total and LDL-cholesterol fell significantly. No significant changes were noted in fecal excretion of neutral and total steroids, or in cholesterol balance comparing probucol and control values.

Since fecal outputs of neutral steroids, dietary cholesterol intake, and percentage of intestinal cholesterol absorption were all determined in ten patients, estimations of daily biliary secretion rates of cholesterol could be calculated as proposed by Vuoristo and Miettinen (44); this technique is based on the assumption that biliary cholesterol is the only source of intestinal cholesterol, and that absorption of endogenous and exogenous cholesterol are roughly equal. Biliary cholesterol secretion for the control period was calculated to be 1226 mg/day ± 209 (SEM) in ten subjects and remained unchanged on probucol treatment (1155 mg/day ± 125). This finding further suggests that biliary cholesterol outputs are not changed by probucol therapy.

DISCUSSION

The purpose of the present study was to examine in some detail the mechanisms for reduction of LDL-cholesterol by probucol. These studies included measurements of various parameters of cholesterol metabolism and estimation of turnover rates of LDL. Our major findings can be discussed.

Plasma lipids

Previous reports indicate that patients with types 2a and 2b hyperlipoproteinemia treated with probucol have reductions in plasma cholesterol concentrations ranging from 10 to 15% (2–15). A similar response was also noted in the present study whether the patients had their plasma cholesterol values elevated or within normal limits. The fall in plasma cholesterol generally was the result of de-

TABLE 5. Fecal steroid excretion during control and probucol-treatment periods, (steady state values)

Patient	Period[a]	Days:No. Determ.[b]	Cholesterol Intake	Total Neutral Steroids	Acidic Steroids	Total Fecal Steroids	Cholesterol Balance
				mg/day ± SEM			
1	I	42:6	98	346 ± 18	277 ± 37	623 ± 36	525 ± 37
	II	35:4	90	573 ± 86[c]	352 ± 60	925 ± 109[c]	735 ± 88
2	I	43:5	116	719 ± 29	458 ± 14	1178 ± 30	1062 ± 30
	II	38:7	121	918 ± 48[c]	533 ± 34	1450 ± 75	1330 ± 74[c]
3	I	45:5	120	943 ± 76	724 ± 40	1666 ± 57	1546 ± 57
	II	43:7	120	675 ± 34[d]	809 ± 52	1421 ± 31[d]	1361 ± 62
4	I	42:5	122	441 ± 21	441 ± 20	882 ± 27	760 ± 28
	II	37:5	164	491 ± 49	389 ± 54	880 ± 90	715 ± 90
5	I	44:7	120	1232 ± 72	678 ± 62	1910 ± 103	1791 ± 101
	II	39:7	156	1110 ± 79	797 ± 53	1907 ± 41	1751 ± 41
6	I	44:3	112	501 ± 40	674 ± 13	1156 ± 62	1043 ± 62
	II	49:3	121	567 ± 45	736 ± 98	1303 ± 143	1182 ± 143
8	I	43:7	99	633 ± 36	563 ± 28	1196 ± 53	1097 ± 53
	II	45:5	108	615 ± 10	541 ± 34	1157 ± 42	1049 ± 42
9	I	41:5	85	408 ± 24	474 ± 40	833 ± 60	798 ± 60
	II	18:3	91	405 ± 27	406 ± 22	810 ± 45	719 ± 48
10	I	14:2	108	949 ± 69	976 ± 22	1925 ± 91	1817 ± 91
	II	42:6	104	964 ± 115	880 ± 41	1844 ± 138	1739 ± 138
11	I	40:5	116	683 ± 21	1005 ± 32	1698 ± 28	1582 ± 28
	II	40:5	116	695 ± 96	1023 ± 23	1718 ± 105	1602 ± 105
12	I	35:3	142	519 ± 69	397 ± 16	916 ± 80	774 ± 80
	II	30:3	142	639 ± 47	575 ± 72	1214 ± 119	1072 ± 119
13	I	43:5	112	423 ± 106	603 ± 36	1028 ± 84	915 ± 84
	II	56:7	112	595 ± 18	501 ± 42	1096 ± 42	984 ± 42
14	I	41:5	121	570 ± 12	496 ± 33	1066 ± 34	945 ± 34
	II	24:3	117	474 ± 28	476 ± 15	950 ± 22	833 ± 22
15	I	46:3	104	816 ± 47	404 ± 121	1220 ± 103	1116 ± 103
	II	14:1	84	777	517	1294	1210
16	I	30:4	128	602 ± 52	812 ± 37	1413 ± 83	1285 ± 83
	II	32:4	128	536 ± 7	956 ± 74	1492 ± 81	1364 ± 81
17	I	31:4	150	651 ± 26	607 ± 21	1258 ± 20	1108 ± 17
	II	28:3	141	501 ± 130	752 ± 86	1253 ± 75	1112 ± 75
Mean ± SEM	I (16)		116 + 4	652 ± 59	599 ± 51	1248 ± 96	1135 ± 95
	II (16)		120 + 6	658 ± 49	640 ± 53	1295 ± 83	1172 ± 85

[a] Period I, control; Period II, probucol.
[b] Days in each period; number of pools analyzed.
[c] Period II significantly higher than Period I by Student's t test, $P < 0.05$.
[d] Period II significantly lower than Period I by Student's t test, $P < 0.05$.

creases in both LDL- and HDL-cholesterol. The former declined by an average of 11%, the latter by 9%. These changes, however, were not consistent for all the patients; a few had no changes in one or another of the fractions.

Most reports (2, 4, 5, 9, 10, 13–15) claim that probucol has little or no effect on plasma triglycerides. The present results are in accord. Overall, no changes were noted for either plasma total TG or VLDL-TG. Furthermore, no alterations were found in plasma postheparin LPL or HTGL. Although a lack of change in lipase activity does not confirm previous reports of reduced activity of LPL in man (6, 20) or in rats (18), it is consistent with a lack of change in triglyceride concentrations.

Cholesterol metabolism

One mechanism whereby probucol might lower plasma cholesterol could be to alter metabolism of cholesterol. Miettinen (6) and Miettinen and Toivonen (7) reported that probucol increases excretion of fecal bile acids and

TABLE 6. Fecal steroid excretion during control and probucol-treatment periods (weekly values immediately after starting probucol)

Patient	Period[a]	Days:No. Determ.[b]	Cholesterol Intake	Total Neutral Steroids	Acidic Steroids	Total Fecal Steroids	Cholesterol Balance
				mg/day			
9	I	41:5	85	408 ± 24	474 ± 40	882 ± 60	798 ± 60
	II	7:1	85	490	521	1011	926
	III	7:1	85	610	433	1043	958
	IV	7:1	85	383	352	735	650
	V	7:1	85	450	376	827	742
	VI	7:1	85	455	446	901	816
	VII	7:1	85	398	369	767	676
14	I	41:5	121	570 ± 12	496 ± 33	1066 ± 34	945 ± 34
	II	7:1	117	392	569	961	844
	III	7:1	117	573	542	1115	998
	IV	7:1	117	510	508	1018	901
	V	7:1	117	462	440	902	785
	VI	7:1	117	426	480	906	789
	VII	7:1	117	471	500	971	854
16	I	30:4	128	602 ± 52	812 ± 37	1413 ± 83	1285 ± 83
	II	7:1	128	1575	863	2438	2310
	III	7:1	128	581	868	1448	1320
	IV	7:1	128	634	1029	1662	1534
	V	7:1	128	563	1010	1573	1445
	VI	7:1	128	551	1078	1629	1501
	VII	7:1	128	531	912	1442	1314
17	I	31:4	150	651 ± 26	607 ± 21	1258 ± 20	1108 ± 17
	II	7:1	141	551	484	1036	895
	III	7:1	141	663	524	1187	1046
	IV	7:1	141	595	383	978	837
	V	7:1	141	609	607	1216	1075
	VI	7:1	141	652	746	1398	1257
	VII	7:1	141	242	903	1145	1004
Mean ± SEM (4)	I		121 ± 13	558 ± 53	597 ± 77	1155 ± 115	1034 ± 105
	II		118 ± 12	752 ± 276	609 ± 86	1362 ± 359	1244 ± 356
	III		118 ± 12	607 ± 20	592 ± 95	1198 ± 88	1081 ± 82
	IV		118 ± 12	531 ± 56	568 ± 157	1098 ± 198	981 ± 192
	V		118 ± 12	521 ± 39	608 ± 142	1130 ± 170	1012 ± 162
	VI		118 ± 12	521 ± 51	688 ± 146	1209 ± 182	1091 ± 174
	VII		118 ± 12	411 ± 62	671 ± 139	1081 ± 143	962 ± 135

[a] Period I, control (mean ± SEM); Period II, first week after starting probucol; Period III, second week after starting probucol; Period IV, third week after starting probucol; Period V, fourth week after starting probucol; Period VI, fifth week after starting probucol; Period VII, sixth week after starting probucol.
[b] Days in each period; number of pools analyzed.

inhibits synthesis of cholesterol, especially during the period when plasma cholesterol is decreasing. Nestel and Billington (16) also found an increase in fecal bile acids in patients treated with probucol. In the current study, determination of cholesterol balance during steady state periods on and off probucol revealed no change in fecal excretion of neutral, acidic, or total steroids.

In addition, balance studies were carried out during the period immediately after starting probucol in four patients. Three of the four showed a small increase in outputs of bile acids during the first week of probucol therapy. This small change seems inadequate to cause a prolonged reduction in plasma levels of LDL and HDL. It could however be secondary to a primary change in lipoprotein metabolism.

Miettinen (6, 7) suggested that probucol causes an initial and transient malabsorption of dietary cholesterol and fat. In our investigation, the absorption of dietary cholesterol was estimated in ten patients by the fecal isotope ratio method of Crouse and Grundy (31). Occasional individuals showed a decrease in absorption, and it is thus possible that the drug has a minor effect on the intestinal phase of cholesterol metabolism. Nonetheless, changes in cholesterol absorption could not be demonstrated for the group as a whole. The differences in the results of the present study and in those of the previous works (6, 7, 16) are unclear but might be due to differences in patient selection. Also, many subjects in the previous studies (6) received 2.0 g of probucol instead of 1.0 g used in the present investigation. Large quantities of probucol re-

maining unabsorbed in the intestinal lumen (45) might have effects that are not seen in smaller doses.

The effects of probucol on biliary lipid metabolism have not been carefully examined previously but a reduction in the lithogenic index of the bile by probucol treatment has been suggested (46). In our study, probucol did not change the composition of fasting gallbladder bile or hepatic secretion rates of biliary lipids. The drug clearly did not increase percentage saturation of bile with cholesterol as does clofibrate (47). It is therefore not surprising that probucol was found not to increase the prevalence of gallstones after several years of treatment with the drug (21).

LDL kinetics

Previous reports on effects of probucol on LDL kinetics have provided conflicting results. Nestel and Billington (16) reported that the FCR of LDL-apoB was increased in four of five patients treated with probucol, although the change for the group as a whole was not statistically significant. Atmeh et al. (48) studied LDL turnover in six patients with Type II hyperlipoproteinemia. In these patients, LDL-cholesterol fell by only 6%, and no significant change could be detected in apoLDL levels. By the same token, they found no significant changes in either synthesis or FCR of apoLDL. In the present study, probucol increased the FCR of apoLDL in eight of nine patients tested, and the increase for the whole group was statistically significant ($P < 0.005$). At the same time, production rates of apoLDL generally were not altered. The major mechanism for LDL lowering by probucol seemingly is to promote the clearance of LDL from plasma.

What is the mechanism for this increased clearance? One possibility is that the drug enhances receptor-mediated removal of LDL. A theoretical basis for such an action has been developed from previous studies in tissue culture. These studies have shown that cells can derive their cholesterol either from newly synthesized cholesterol or from LDL through receptor-mediated endocytosis. The synthesis of LDL receptors appears to be linked closely to cholesterol synthesis, and more specifically to the rate of formation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). Under circumstances where the synthesis of HMG-CoA reductase is increased, the synthesis of LDL receptors also is enhanced. This is illustrated by two modes of therapy. First, bile acid sequestrants increase the conversion of cholesterol into bile acids; this stimulates the synthesis of HMG-CoA reductase, and LDL receptor number increases. This sequence seemingly accounts for the LDL-lowering action of bile acid sequestrants (49, 50). Furthermore, competitive inhibitors of HMG-CoA reductase activity—compactin and mevinolin—also stimulate synthesis of HMG-CoA reductase and LDL receptors (51); these agents likewise are potent LDL-lowering drugs (52, 53).

Could probucol also increase receptor-mediated catabolism of LDL in the liver? If so, it too would have to alter the metabolism of cholesterol or bile acids. Such an effect could not be detected in our study. Excretions of neither cholesterol nor bile acids were enhanced. Neither were excretions of fecal steroids reduced; thus the drug did not grossly inhibit cholesterol synthesis. Therefore, our findings do not support an action on cholesterol synthesis and thus on receptor-mediated clearance of LDL. Nevertheless, it is theoretically possible that probucol might be a weak inhibitor of HMG-CoA reductase leading to an increased production of this enzyme and enhanced LDL receptor formation; a compensatory rise in HMG-CoA reductase might overcome a block in cholesterol synthesis to restore production to normal and still maintain an increased number of LDL receptors.

Another possibility is that probucol becomes incorporated into the LDL particle, alters the configuration of surface apoproteins, and thereby increases the affinity of LDL for its receptor. The observation that probucol in plasma is present in lipoproteins (54) is consistent with this possibility. Finally, the drug might promote uptake of LDL by the non-receptor pathway. For example, the drug has been reported to lower plasma cholesterol in patients with homozygous familial hypercholesterolemia (55). These patients have no LDL receptors (56, 57). If the drug promotes LDL clearance in such patients, it likely occurs via the receptor-independent pathway. To be certain, however, it will be necessary to compare the two pathways of LDL clearance by a recently described technique employing isotope kinetics (58).

Conclusions

From this study several conclusions might be drawn. Probucol causes a moderate reduction in plasma total cholesterol, and the major decrease occurs in the LDL fraction. The lowering of LDL is due to enhanced clearance, and not to decreased synthesis of this lipoprotein. Unfortunately, probucol also lowers HDL-cholesterol, which is of concern in view of the association between low HDL and risk of coronary heart disease.

Because of the limited potency of probucol for LDL lowering, its value as single-drug treatment of significant hypercholesterolemia can be questioned. However, because of its apparent lack of side effects, such as increased saturation of bile, the agent may be useful in multiple-drug therapy; the use of two or more agents in combination appears to be the most effective means of treatment of severe hypercholesterolemia (59–61), and probucol could be considered as one such drug.

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