Effect of Probucol, Pantethine and Their Combinations on Serum Lipoprotein Metabolism and on the Incidence of Atheromatous Lesions in the Rabbit

Katsuhiko TAWARA, Masanao ISHIHARA, Hidemasa OGAWA
and Munehiro TOMIKAWA

Research Institute, Daiichi Seiyaku Co., Ltd., 16-13, Kitakasai 1-chome,
Edogawa-ku, Tokyo 134, Japan

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Abstract—Effect of probucol, pantethine and their combinations on serum lipoprotein metabolism and on the incidence of atheromatous lesions in aorta and coronary artery was studied in cholesterol-fed rabbits. Probucol treatment (0.5% in diet) resulted in reducing HDL cholesterol and serum apo A-I levels significantly, while pantethine treatment (0.25%–0.75% in diet) tended to increase HDL cholesterol and serum apo A-I levels. Combined treatment with these two drugs showed a significant prevention in the reduction of HDL cholesterol and serum apo A-I levels by probucol alone. Probucol or pantethine treatment reduced effectively (V)LDL cholesterol and serum apo B levels, and these effects were accelerated additively when the two drugs were given concurrently. Atheromatous lesions in aorta and coronary artery in cholesterol-fed rabbits were prevented by the treatment with probucol (0.5% in diet) or pantethine (0.75% in diet) for 24 weeks. The combined treatment with these two drugs showed more marked prevention than either drug alone. From these findings, it is concluded that the combined treatment of probucol with pantethine is effective for improvement of serum lipoprotein disorders and for prevention of the incidence of atheromatous lesions in aorta and coronary artery in cholesterol-fed rabbits.

Elevated cholesterol level, specifically high level of low density lipoprotein (LDL) cholesterol in the blood stream, is believed to be one of the major causes of developing atherosclerotic disease, especially of the coronary artery (1–4). On the other hand, the inverse relationship between plasma levels of high density lipoprotein (HDL) and risk of coronary artery disease have been demonstrated in numerous studies (5–9).

Probucol treatment induces a marked reduction in total cholesterol and LDL cholesterol levels (10–15) concomitant with a significant reduction in HDL cholesterol and apo A-I levels in serum (16, 17).

If the reduction of HDL cholesterol and serum apo A-I levels by probucol could be prevented by the combination with other agents such as HDL cholesterol elevating

agents, the hypocholesterolemic action of probucol might be more useful for the prevention of atherosclerotic lesion.

From this point of view, combined treatment with probucol and pantethine on serum lipids, lipoproteins and apolipoproteins were studied in cholesterol-fed rabbits in the present study, because pantethine elevated effectively HDL cholesterol and apo A-I levels in serum as reported previously (18). Combined treatment with both agents on the incidence of atheromatous lesions in the aorta and coronary artery was also examined.

Materials and Methods

Treatment of animals: Male Japanese albino rabbits weighing 2.0–2.5 kg (Kanamaru Laboratory Animal Center, Tokyo) were divided at random into 9 groups of 8 animals

each after a 2-week acclimation period with normal standard laboratory chow (Oriental RC-4, Oriental Co., Tokyo). The 9 groups were treated for 9 weeks, respectively, with 1) normal standard laboratory chow (group I), 2) 0.5% cholesterol diet (group II), 3) 0.5% cholesterol diet + 0.25% pantethine (group III), 4) 0.5% cholesterol diet + 0.5% pantethine (group IV), 5) 0.5% cholesterol diet + 0.75% pantethine (group V), 6) 0.5% cholesterol diet + 0.5% probucol (group VI), 7) 0.5% cholesterol diet + 0.5% probucol + 0.25% pantethine (group VII), 8) 0.5% cholesterol diet + 0.5% probucol + 0.5% pantethine (group VIII) and 9) 0.5% cholesterol diet + 0.5% probucol + 0.75% pantethine (group IX) (Fig. 1). Rabbits were received 150 g of diet per day and were allowed ad libitum water. Blood samples to determine serum lipids, lipoproteins and apolipoproteins were taken from the ear vein before and after 2, 4, 6 and 9 weeks of treatment. Histopathological examination on arterial atheromatous lesions in cholesterol-fed rabbits in groups II, V, VI and IX (7 animals in each group) was carried out after a 24-week treatment.

Chemicals: Probucol (Lot No.: MM810519, Dow Chemical-Japan, Tokyo) and pantethine (Lot No.: 272, Daiichi Seiyaku Co., Tokyo) were used. Other chemicals used

were of reagent grade.

Determination of serum lipids and lipoprotein cholesterol: Serum cholesterol, phospholipids and triglyceride were determined by using enzymatic test kits: Daitest CHO-2 (Daiichi Pure Chemicals Co., Tokyo), Phospholipid B Test Wako (Wako Junyaku Co., Tokyo) and Triglyceride C-II Test Wako (Wako Junyaku Co., Tokyo) respectively. Serum lipoprotein fractions were prepared by the ultracentrifugation method (18). Serum samples of 150 μ l each were adjusted to a density of 1.063 by adding 20 μ l of KBr solution ($d=1.491$) to centrifuge tubes (0.175 CN tubes, Hitachikoki, Co., Tokyo). The mixture was centrifuged in an RPL-42T-101 rotor (Hitachikoki, Co., Tokyo) at $128,100 \times g$ for 16 hr at 16°C and the 110 μ l bottom fraction (HDL) was collected for determination of cholesterol. Cholesterol values in the VLDL+LDL [(V)LDL] fraction were calculated from the difference between total serum cholesterol and HDL cholesterol values.

Determination of serum apolipoproteins: Serum apo A-I and apo B levels were determined by the rocket technique of Laurell (19). One percent agarose gel containing 5% dextran was prepared in barbital buffer ($\mu=0.05$, pH 8.6) with 5% anti A-I serum for apo A-I determination. For apo B

Fig. 1. Experimental schedule

determination, 0.75% agarose gel containing 5% dextran was prepared in barbital buffer ($\mu=0.025$, pH 8.6) with 5% anti B serum. The electrophoresis was performed in an electrophoresis apparatus (Marisol Co., Tokyo) and ran for 16 hr at 4°C at a field strength of 7V/cm (50V). Samples and the serially diluted serum as reference were applied on the plate. The apolipoprotein concentrations in sera were expressed in mg/dl.

Pathology: After treatment for 24 weeks, rabbits in groups II, V, VI and IX (7 rabbits in each group) were killed by exsanguination under pentobarbital-Na anesthesia (40 mg/kg, i.v.), and the entire aorta and heart were removed. Aorta free of adventitial fat was opened longitudinally and stained with Oil-red O and photographed to determine the extent of atherosclerotic lesions. The degree of the lesions was graded on a 0–4 scale according to Duff et al. (20). Tissues (ascending and middle portion of abdominal aorta and heart) were fixed with neutral buffered formalin and then calcified and embedded in paraffin blocks. Cross sections (5–6 μ m thick) were stained with hematoxyline and eosin (H-E).

Analysis of data: Statistical significance of data was analyzed by Student's *t*-test. Data are presented as the mean $\pm$ S.E.

Results

Serum lipids and lipoprotein cholesterol: As shown in Table 1, Cholesterol feeding resulted in a great increase in total and (V)LDL cholesterol levels in serum depending on the period. The highest value was observed at the 6th week (103 and 593 fold of normal rabbits, respectively). In contrast, HDL cholesterol level was not significantly affected by cholesterol feeding. Phospholipids levels in serum were also increased (7.5-fold of normal rabbits at the 9th week). Triglyceride level in serum was slightly increased.

In the pantethine-treated groups (groups III, IV and V), total and (V)LDL cholesterol together with phospholipids levels in serum were almost dose-dependently decreased, while HDL cholesterol level was moderately elevated at the 6 and 9th week, and serum triglyceride level was also reduced by only

treatment with higher doses.

In the probucol-treated group (group VI), a marked reduction of total and (V)LDL cholesterol levels concomitant with the decrease in HDL cholesterol were observed at all points of measurement during the 9 week period. Phospholipids levels in serum were also decreased but triglyceride level was not affected.

The combined treatment with probucol and pantethine (groups VII, VIII and IX) caused a additive effect in lowering the total and (V)LDL cholesterol levels in serum at all the examined periods ($\sim$ 9 weeks), while the reduction of HDL cholesterol level by probucol treatment alone was significantly prevented in a dose-dependent manner by the combination with pantethine. Serum phospholipids levels were also additively reduced by the combinations; however, serum triglyceride level was decreased with no further improvement over the pantethine or probucol treatment alone.

Serum lipids and lipoprotein cholesterol levels in groups II, V, VI and IX at the 18 and 24th week are summarized in Table 2. Almost the same results as seen at the 9th week were observed.

Serum apolipoproteins: The high cholesterol diet also caused a marked increase in the concentration of serum apo B (about 10-fold that of normal rabbits, Fig. 2); however, the concentration of serum apo A-I was moderately decreased (about 60% of normal rabbits, Fig. 3) by feeding the cholesterol-supplemented diet for 9 weeks.

Pantethine or probucol treatment reduced serum apo B levels effectively, and these effects were enhanced additively by the combination with both agents (Fig. 2).

Pantethine treatment tended to increase serum apo A-I levels; on the contrary, probucol treatment caused a significant reduction of it (about 60%). Combined treatment with these two drugs showed a significant prevention in the reduction of serum apo A-I levels by probucol treatment alone (Fig. 3).

Histopathological observations: The protective effect of pantethine (0.75% in diet), probucol (0.5% in diet) and their combination (0.75% pantethine + 0.5% probucol in diet) was studied on the 24th week atherosclerotic

Table 1. Effect of probucol, pantethine and their combinations on serum lipids and lipoprotein cholesterol levels in cholesterol-fed rabbits

Treatment	Serum lipids (mg/dl)	Time of treatment (weeks)				
		0	2	4	6	9
Control	Total cholesterol	21.1±1.3	788.3±138.6	1005.4±122.1	1920.0±285.1	1680.3±252.3
	(V)LDL cholesterol	5.0±0.6	769.4±139.8	980.9±122.7	1897.6±286.2	1658.9±253.6
	HDL cholesterol	16.1±0.8	18.9±2.3	24.5±1.6	22.4±2.4	21.4±2.3
	Phospholipids	70.9±4.2	310.4±51.2	411.4±71.3	453.1±66.1	464.9±69.0
	Triglyceride	111.4±8.0	144.4±10.5	154.7±16.6	177.4±9.8	123.8±9.3
Pantethine (0.25%)	Total cholesterol	20.3±1.9	627.9±84.0	744.9±126.8	1002.0±252.3*	1268.1±235.9
	(V)LDL cholesterol	4.6±0.8	612.5±84.2	720.6±125.0	975.6±249.7*	1242.2±235.6
	HDL cholesterol	15.7±1.3	15.5±1.5	24.3±2.6	26.4±2.9	25.8±1.4
	Phospholipids	74.1±4.3	299.5±33.4	354.0±59.2	269.4±53.2*	374.7±70.3
	Triglyceride	106.5±5.5	161.6±17.9	139.9±13.2	146.0±25.7	137.2±40.1
Pantethine (0.5%)	Total cholesterol	22.3±1.8	602.1±94.3	544.6±81.8**	971.0±113.4*	1141.5±125.9
	(V)LDL cholesterol	5.3±0.6	578.2±93.0	531.2±83.2*	948.3±113.1*	1119.6±125.7
	HDL cholesterol	17.0±1.6	23.9±3.7	23.4±2.3	22.7±0.9	22.0±0.8
	Phospholipids	73.4±3.3	271.0±35.5	217.2±25.1*	285.4±17.3*	346.2±25.5
	Triglyceride	105.2±6.8	158.5±21.4	91.9±8.4**	122.4±6.1**	136.3±20.6
Pantethine (0.75%)	Total cholesterol	21.7±1.3	518.2±77.3	419.5±105.1**	698.5±94.8**	492.3±65.4**
	(V)LDL cholesterol	4.6±0.6	501.1±77.1	396.3±105.2**	670.5±95.0**	468.0±65.4**
	HDL cholesterol	17.1±1.3	17.1±1.3	23.2±2.5	28.0±0.7*	24.3±1.8
	Phospholipids	74.6±3.4	222.5±24.7	219.2±31.5*	202.5±18.6**	183.9±16.6**
	Triglyceride	107.5±7.7	126.6±12.0	126.6±21.4	130.3±4.0**	70.3±6.5***
Probucol (0.5%)	Total cholesterol	20.7±1.2	488.0±44.1	691.7±118.4	550.5±145.6**	685.7±105.3**
	(V)LDL cholesterol	5.0±0.7	480.7±44.3	682.3±118.6	539.1±146.3**	676.8±105.1**
	HDL cholesterol	15.7±1.3	7.3±0.4***	9.4±1.0***	11.4±1.5**	8.9±0.5***
	Phospholipids	72.2±5.4	237.1±22.3	313.1±55.1	208.2±45.1**	230.9±22.7*
	Triglyceride	103.0±6.7	162.0±23.2	189.2±22.8	171.1±29.5	101.1±15.9

Table 1. continued

Treatment	Serum lipids (mg/dl)	Time of treatment (weeks)				
		0	2	4	6	9
Probucol (0.5%) +Pantethine (0.25%)	Total cholesterol	20.6±2.0	420.6±39.6*	408.2±73.4**	496.3±121.8**	644.2±84.3**
	(V)LDL cholesterol	4.8±0.6	412.5±39.5*	394.5±73.7**	482.2±123.1**	630.7±84.6**
	HDL cholesterol	15.7±1.7	8.1±0.2**	13.7±0.7****	14.1±1.8*	13.5±1.7**
	Phospholipids	77.5±4.5	226.8±12.9	200.5±17.0*	154.1±24.2**	239.5±18.8*
	Triglyceride	102.3±6.1	159.6±17.6	160.9±29.5	118.5±9.7***	158.9±27.2
Probucol (0.5%) +Pantethine (0.5%)	Total cholesterol	20.8±1.4	283.7±59.2**	260.5±58.1****	462.3±85.9**	565.4±70.8**
	(V)LDL cholesterol	5.2±0.7	272.7±58.9**	237.6±59.0****	446.1±85.0**	541.7±69.6**
	HDL cholesterol	15.6±1.2	11.0±1.0**	22.9±2.4***	16.2±1.3*	23.7±1.9***
	Phospholipids	72.0±4.9	176.8±19.9*	167.5±22.0**	153.0±20.9**	192.0±16.2**
	Triglyceride	102.2±5.5	160.8±27.6	137.5±19.4	114.1±13.3**	95.0±4.5*
Probucol (0.5%) +Pantethine (0.75%)	Total cholesterol	22.5±2.2	187.4±37.0****	153.1±37.9****	370.8±39.6**	388.6±58.9***
	(V)LDL cholesterol	5.8±0.9	175.6±37.1****	129.6±38.1****	352.3±39.7***	361.4±58.7***
	HDL cholesterol	16.7±1.6	11.8±1.3*	23.5±1.1***	18.4±0.8**	27.3±2.1***
	Phospholipids	74.3±3.3	149.0±17.4**	114.3±14.5****	137.4±17.6**	149.6±14.9***
	Triglyceride	103.1±7.1	140.0±21.8	89.4±12.8****	101.0±15.9**	84.7±11.7*
Normal	Total cholesterol	22.2±1.9	25.8±2.2****	27.1±2.4****	18.6±1.8****	23.1±2.3****
	(V)LDL cholesterol	5.5±0.8	9.4±2.2****	7.6±2.0****	3.2±0.6****	5.7±0.7****
	HDL cholesterol	16.7±1.4	16.4±0.8***	19.5±1.1***	15.4±1.3	17.5±1.7**
	Phospholipids	73.0±4.6	74.5±2.9****	78.0±6.5****	62.4±4.7****	61.6±4.3****
	Triglyceride	103.6±8.0	112.2±14.5	57.1±7.3***	60.2±4.7****	48.0±3.8****

The values are the mean±S.E. of 8 rabbits. *P<0.05, **P<0.01, ***P<0.001 vs. respective Control. *P<0.05, **P<0.01, ***P<0.001 vs. respective Probucol.

Table 2. Effect of probucol, pantethine and their combination on serum lipids and lipoprotein cholesterol levels in cholesterol-fed rabbits (at the 18th and 24th week)

Treatment	Cholesterol (mg/dl)			Phospholipids (mg/dl)	Triglyceride (mg/dl)
	Total	(V)LDL	HDL		
18th week					
Control	1848.6±141.2	1830.3±140.6	18.3±1.7	607.2±43.4	171.2±10.6
Pantethine (0.75%)	894.6±156.2***	875.9±155.0***	18.8±1.5	304.2±49.1***	110.6±15.4**
Probucol (0.5%)	1080.3±173.3**	1070.6±173.4**	8.9±0.7***###	386.0±47.3**	164.5±23.4
Probucol (0.5%) + Pantethine (0.75%)	587.1±111.4***	571.7±111.7***	15.4±1.3	238.0±36.2***	113.6±15.7*
24th week					
Control	2337.9±346.3	2320.6±346.6	17.3±1.9	776.4±97.8	369.2±30.8
Pantethine (0.75%)	1469.3±74.9*	1453.1±74.9*	16.3±1.0	478.9±27.8*	191.2±17.4***
Probucol (0.5%)	1380.8±153.9*	1371.2±154.8*	9.6±1.0***##	628.3±68.1	520.6±50.4*###
Probucol (0.5%) + Pantethine (0.75%)	820.5±85.6***###	809.8±85.6***###	10.7±1.0*##	357.6±42.7** #	223.4±32.8**

The values are the mean±S.E. of 7 rabbits. *P<0.05, **P<0.01, ***P<0.001 vs. respective Control. #P<0.05, ##P<0.01, ###P<0.001 vs. respective Probucol. #P<0.05, ##P<0.01, ###P<0.001 vs. respective Pantethine.

lesions in the aorta and coronary artery of the 4 groups (II, V, VI and IX).

Gross findings: Figure 4A shows the atheromatous lesions in the aorta of the control rabbits (group II). The findings are

Fig. 2. Effect of probucol, pantethine and their combinations on serum apo B levels in cholesterol-fed rabbits (at 9th week). Each column represents the mean $\pm$ S.E. of 8 rabbits. *P<0.05, **P<0.01 vs. control.

Fig. 3. Effect of probucol, pantethine and their combinations on serum apo A-I levels in cholesterol-fed rabbits (at 9th week). Each column represents the mean $\pm$ S.E. of 8 rabbits. *P<0.05 vs. control.

characterized as an advanced atherosclerosis, consisting of raised form cell lesions and some plaques covering the entire surface of the artery. Table 3 summarizes the effect of pantethine, probucol and their combination on the extent of aortic lesions after 24 weeks of treatment. Pantethine (group V) or probucol (group VI) was found to be effective in preventing the progression of atheromatous lesions in the aorta. The combined treatment with pantethine and probucol (group IX) was much more effective than either drug alone (Fig. 4B).

Microscopic findings: Figure 5A shows typical atherosclerotic lesions in the ascending portion of the control rabbit aorta in group II, consisting of intimal thickening with accumulation of foam cells, fibrotic cell proliferation, fragmentation of elastic laminae,

Fig. 4. Gross findings in arterial atheromatous lesions (Oil-red O stain). (A) Control group (B) Probucol (0.5%) plus pantethine (0.75%) treated group.

Table 3. Effect of probucol, pantethine and their combination on the incidence of aortic atheromatous lesions in cholesterol-fed rabbits (gross findings, at the 24th week)

Treatment	No. of rabbits	Degree of aortic lesion					
		0	1	2	3	4	Mean±S.E.
Control	7	0	0	0	2	5	3.7±0.2
Pantethine (0.75%)	7	0	2	3	2	0	2.0±0.3***
Probucol (0.5%)	7	1	2	2	2	0	1.7±0.4**
Probucol (0.5%) +Pantethine (0.75%)	7	4	2	1	0	0	0.6±0.3***##

P<0.01. *P<0.001 vs. Control. P<0.05 vs. Probucol ##P<0.01 vs. Pantethine

A **A** **B** **B**

Fig. 5. Histological findings in ascending aorta (H.E. stain ×50). (A) Control group (B) Probucol (0.5%) plus pantethine (0.75%) treated group.

Fig. 6. Histological findings in coronary artery (H.E. stain ×50). (A) Control group (B) Probucol (0.5%) plus pantethine (0.75%) treated group.

lipid deposition both intra-cellularly and in the extra-cellular space and superficial fibrous capsules in the sections stained with hematoxyline and eosin.

Pantethine or probucol treatment was found to be effective in preventing the

progressin of the atheromatous lesions, i.e., less marked accumulation of the foam cells and inhibition of fibrous cell proliferation in intima and medial cells was observed (photographs not shown). The combined treatment with pantethine and probucol was

Fig. 7. Effect of probucol, pantethine and their combination on the incidence of atheromatous lesions in aorta and coronary artery in cholesterol-fed rabbits (histological findings, at the 24th week). Each column represents the mean value from 7 rabbits. Prob: probucol (0.5%), PaSS: pantethine (0.75%), PaSS+Prob: pantethine (0.75%) plus probucol (0.5%).

found to be more effective in preventing the progression of atheromatous lesions in aorta. Only a slight intimal thickening and lipid deposition was observed (Fig. 5B).

Figure 6A shows the lesion of coronary artery in the cholesterol-fed control rabbits (group II). The arterial lumen was greatly narrowed by intimal thickening with accumulation of foam cells and were noted by degeneration of medial smooth muscles and fragmentation of elastic fibers.

Pantethine or probucol treatment resulted in a significant reduction of intimal thickening and a weak foam cell accumulation (Fig. 7, photographs not shown). The combined treatment with pantethine and probucol (group IX) resulted in a highly significant reduction of the degree of the intimal thickening with foam cell accumulation and was found to be highly effective in preventing the progression of atheromatous lesions in the coronary arteries (Figs. 6B and 7).

Discussion

Associations between low levels of HDL and the risk of developing atherosclerotic disease, specifically of the coronary arteries, have been demonstrated in numerous studies (5–9). However, there were few reports

about the fact that lowered HDL cholesterol levels in serum accelerated the progression of atherosclerosis in animal models. In clinical study, a hypolipidemic agent that lowered HDL cholesterol prevented the incidence of coronary arterial diseases (21). More detailed epidemiological studies on a large scale are required to elucidate the relationship between the HDL level and the incidence of atherosclerotic diseases.

Recently, the relationship of the reduction in the incidence of coronary heart disease to the lowering of serum total and LDL cholesterol was clarified in large scale epidemiological studies (22, 23).

From the viewpoint of the physiological action of HDL, i.e., removal of stored cholesterol in peripheral tissues, including the aortic wall, to the liver, it might be more effective if the lowering activity of probucol on HDL cholesterol and serum apo A-I levels could be compensated by some means. The present study was carried out with two major objectives (a) to clarify whether the reduction of HDL cholesterol and serum apo A-I levels by probucol treatment could be compensated by the combination with other agents such as HDL-elevating agents like "pantethine", an intermediate precursor of coenzyme A. (b) to clarify whether the improvement of serum

lipoprotein disorders could have a favorable influence on arterial atheromatous lesions.

In the present study with hyperlipidemic rabbits, probucol treatment (0.5% in diet) resulted in a marked reduction in serum total and (V)LDL cholesterol levels. A significant reduction of HDL cholesterol and serum apo A-I levels was also observed in probucol treated rabbits. On the other hand, pantethine treatment (0.25%–0.75% in diet) caused a dose-dependent reduction in serum total and (V)LDL cholesterol levels. HDL cholesterol and serum apo A-I levels were moderately elevated in this experiment with little difference from that of the previous report (18). The combination of probucol with pantethine resulted in a synergistic effect in lowering the serum total and (V)LDL cholesterol levels, while their combinations exerted a significant dose-dependent prevention for the decrease of HDL cholesterol and serum apo A-I levels by probucol treatment alone (Table 1 and Fig. 3).

In clinical studies, several combination therapies with different types of hypolipidemic agents have been reported. Cholestylamine and probucol combination therapy in familial hypercholesterolemia can result in a greater reduction in serum cholesterol than with either drug alone, but failed to compensate for the reduction of HDL cholesterol by probucol (24). Mizuno et al. (25) reported that a combined administration of probucol and pantethine in heterozygous familial hypercholesterolemia was effective in preventing the HDL cholesterol reduction by probucol in addition to synergistic decrease in serum cholesterol.

It has been reported that probucol reduced HDL cholesterol (apo A-I) by inhibiting the biosynthesis of apo A-I in the organs from the kinetic study of labelled HDL (26), although the detailed mechanism of probucol with regards to the decrease in HDL has not been clear yet.

On the other hand, pantethine increased HDL cholesterol through the stimulation of the VLDL-HDL pathway, i.e., the heparin-releasable lipoprotein lipase activity in the epididymal adipose tissue as well as the lecithin: cholesterol acyltransferase (ACAT) activity in serum was markedly increased by

treatment with pantethine (27). Pantethine treatment also resulted in an acceleration of the biosynthesis of apo A-I in the perfusion system of isolated rabbit livers (28). Therefore, it has been speculated that these opposite actions of the combined drugs on HDL cholesterol and apo A-I might contribute to their compensatory actions.

In this study, the severity of atheromatous lesions in aorta and coronary arteries in cholesterol-fed rabbits was effectively prevented by probucol or pantethine (Fig. 7). These findings are consistent with the results of Krichevsky et al. (29) in the probucol study and with the results of Carrara et al. (30) in the pantethine study.

The protective effect of pantethine may be possibly due to the increased HDL cholesterol levels and the marked reduction in the serum levels of atherogenic (V)LDL. Pantethine also has other actions, i.e., increasing the arterial cholesterol ester hydrolase activities (31). It may be related to the protective effect of pantethine on atheromatous lesions in the artery.

Protective effect of probucol may be related mainly to the marked reduction of atherogenic (V)LDL in the serum. The reduction of HDL cholesterol may be less important than the reduction of (V)LDL cholesterol for the progression of atheromatous lesions in this animal models, because (V)LDL cholesterol level in the cholesterol-fed rabbit was extremely high, and HDL cholesterol level was extremely low. There is another possible action for the protective effect of probucol in the progression of atherosclerosis: it has the advantage of inhibiting the oxidation of LDL in cultured endothelial cells and macrophages (32). Combined treatment with probucol and pantethine exerted a synergistic effect in preventing the progression of arterial atheromatous lesions both in aorta and in coronary arteries (Fig. 7). This marked protective effect in their combination therapy would be related to the cumulative actions of these two agents mentioned above.

In conclusion, the combined treatment with probucol and pantethine has a favorable influence on the metabolism of serum lipoproteins and has a protective effect in the

progression of atheromatous lesions in the aorta and coronary arteries in cholesterol-fed rabbits.

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