PHARMACOLOGICAL STUDY OF HYDROXY BIS-[2-(P-CHLOROPHENOXY)-ISOBUTYRIC ACID] ALUMINIUM
[II] THE EFFECT OF HYDROXY BIS-[2-(P-CHLOROPHENOXY)-ISOBUTYRIC ACID] ALUMINIUM ON LIPID METABOLISM
IN HYPERCHOLESTEROLEMIC RATS

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Abstract—The effect of alfibrate on the lipid metabolism in serum and liver was studied in the rats maintained on a cholesterol diet with simultaneous oral administration of alfibrate (150–900 mg/kg, daily) for two weeks. Clofibrate was used as a reference. The growth in alfibrate group was less inhibited than in clofibrate group and the increase in liver weight was 7% for alfibrate and 39% for clofibrate at 150 mg/kg dosage. Alfibrate inhibited the elevation of serum cholesterol by 60–67%, which is almost the same level of inhibition as for clofibrate. About 70% of inhibition in serum β -lipoprotein level, 36–58% decrease in the ratio of total cholesterol to phospholipids and a significant decrease in the ratio of free to esterified cholesterol were observed in alfibrate treated rats. About 15–23% inhibition in serum triglycerides and almost the same level of inhibition in serum phospholipids was observed, the rate of inhibition by alfibrate being less than that by clofibrate. Alfibrate administration produced no significant change in liver cholesterol concentration in the hypercholesterolemic rats, but caused a 40–50% decrease on liver triglyceride and free fatty acid concentration, and 9 and 22% increase in phospholipid concentration at low and high dosages respectively. Any detectable change in total lipid concentration on liver, brain, kidneys, testes and the weight of epididymal fat tissues was not observed with alfibrate administration. Palmitic acid in cholesterol ester fraction of both liver and serum was decreased significantly, and in general, oleic acid tended to increase with alfibrate.

In a previous paper (1), we have reported the effect of hydroxy bis-[2-(p-chlorophenoxy)-isobutyric acid] aluminium (alfibrate) on the lipid metabolism in normal rats and have found that it has a potent effect on lowering cholesterol and triglyceride level in liver and serum, comparable with the effect of ethyl chlorophenoxy isobutyrate (clofibrate) employed as a reference. In this paper, the alfibrate effect has been studied in hypercholesterolemic rats induced by feeding a synthetic diet containing 1% cholesterol and 0.5% Bacto oxgal.

In providing a cholesterol diet to rats for two weeks, cholesterol, triglyceride and free fatty acid levels in serum were elevated to 5.6, 1.5 and 2.5 times those of normal rats respectively, and phospholipid level was decreased by 28%, while in the liver, 15 times

the cholesterol, 5 times the triglycerides and twice the fatty acids of normal rats accumulated without any change in the phospholipids. In contrast to the results in normocholesterolemic rats, alfibrate exerted a greater effect on serum cholesterol than serum triglycerides in the hypercholesterolemic rats. Lipid content in some organs and fat tissues was also examined as it was suspected that the divergence of lipids to peripheral tissue could occur as a possible mechanism partly responsible for the hypolipidemic effect.

At the same time the esterified fatty acids in each lipid fraction were analyzed by gas chromatography to determine the qualitative as well as the quantitative effect of alfibrate on the lipids.

MATERIALS AND METHODS

Materials

Bacto oxgal was obtained from Difco Laboratories, Kieselgel H from Merk, Rhodamin 6 G, Tokyo Kasei company, borontrifluoride-methanol and standards for cholesterol oleate, triolein, oleic acid were all purchased from Applied Sciences. Diethylene glycol succinate (15%) on Celite 545, acid washed, 60~80 mesh, were products of Shimazu Seisakusho LTD.

Experimental animals

Three groups of Wistar strain male rats weighing 110–130 g were fed the synthetic cholesterol diet containing 1% cholesterol and 0.5% Bacto oxgal in sucrose base as shown

TABLE 1. Composition of cholesterol diet containing 1% cholesterol.

Cholesterol		1	
Bacto oxgal		0.5	
Milk casein		10	
Salt mix*		4	
Shortening oil		15	
Choline chloride		0.2	
PABA		0.1	
Inositol		0.1	
Vitamine mix**			
Sucrose		67.6	
Total		100 g	
** Vitamine mix/kg		Salt mix*	
B ₁	5 mg	CaCO ₃	30 g
B ₆	5 mg	K ₂ HPO ₄	32.5
PaCa	20 mg	CaHPO ₄ ·2H ₂ O	7.5
Biotin	100 µg	MgSO ₄ ·7H ₂ O	10.2
B ₂	6 mg	NaCl	16.8
B ₁₂	10 µg	Fe(C ₆ H ₅ O ₇) ₂ ·6H ₂ O	2.8
Folic acid	500 µg	KI	0.08
Niacin	20 mg	MnSO ₄ ·4H ₂ O	0.5
D ₂	3000 IU	ZnCl ₂	0.025
A	30000 IU	CuSO ₄ ·5H ₂ O	0.03
K ₃	5 mg		

in Table 1. From the beginning of the experiment, 150–900 mg/kg alfibrate suspended in 1% tragacanth solution was orally administered every day for two weeks to the first group, 150–300 mg/kg of clofibrate emulsified in 0.5% methylcellulose solution to the second group as a reference and saline to the third group as a control. At the termination of experiment the animals were lightly anesthetized with ether after being fasted for 24 hr. Lipid extraction from tissues and determination of cholesterol, triglycerides, free fatty acids and phospholipids in serum and liver were carried out as described in a previous paper (1). Total lipid concentration in fat tissue and several organs was measured by the method of Amenta (2). Serum β -lipoprotein was estimated using the Wako β -lipo kit.

Thin layer chromatography of the lipids

The procedure used was generally that of Mangold (3). Solvent of the lipid extracts was evaporated under vacuum and the residue was dissolved in a small amount of hexane. The lipid in hexane was applied on thin layer plate (0.075 cm thick) of Kieselgel H activated at 110° for 1 hr before use, and the plate was developed by the petroleum ether: ether: acetic acid (70:30:1) at 20°. Spraying 0.1% Rhodamin 6G solution in 95% ethyl alcohol over the plates visualized the spots under ultraviolet lamp (366 m μ), and each spot corresponding cholesterol esters, triglycerides and free fatty acid were scraped off to be collected into a small chromatography tubes with fused in fitted disc and extracted with 3 ml of ether.

Gas chromatography of fatty acid methyl esters

The fractions of free fatty acids, and cholesterol esters and triglycerides previously hydrolyzed with 0.5 N KOH in methylalcohol at 100° for 5 min, were heated with 14% boron trifluoride in methyl alcohol at 100° for 2 min. Fatty acid methyl esters thus obtained were extracted with petroleum ether (bp. 40°–60°) and washed with water. The methyl esters dissolved in a small amount of hexane were analyzed by Shimazu Gas Chromatograph, Model GC-4A under the following conditions, detector: flame ionization, 210°, column: diethyleneglycol succinate (DEGS) 15% on Celite 545, acid, washed, 60~80 mesh, 185°, injector: 210°, carrier gas flow: 40 ml/min (N₂).

RESULTS

Effect of alfibrate on growth rate and liver weight

The growth of rats maintained on the cholesterol diet appeared to be slightly inhibited in the alfibrate and clofibrate-treated groups, and 300 mg/kg administration of the latter resulted in an especially low growth rate as shown in Table 2. The liver was enlarged by the drug treatment, 7% increase in alfibrate group and 39% increase in clofibrate group at 150 mg/kg dosage, and the magnitude of liver enlargement became greater as the dosage increased and clofibrate produced more severe hepatomegaly than alfibrate. As compared to normal rats, the magnitude of hepatomegaly was rather little in rats on the cholesterol diet, no doubt due to the increased liver size arising from cholesterol fatty liver in the control group.

TABLE 2. Effect of alfibrate on growth rates and liver size in hypercholesterolemic rats.

Drug administered (mg/kg daily)	No. of rats	Rate of increase in body weight (as % of 0 day) mean $\pm$ S.E.		Liver size	
		0 week (average B.W.)	2 weeks	Liver wt(g)/100 g rat wt. mean $\pm$ S.E.	% increase
None	7	100 (108g)	112 $\pm$ 3.3	4.16 $\pm$ 0.087	100
Alfibrate	150	9	100 (106g)	4.36 $\pm$ 0.186	107**
	300	8	100 (107g)	4.37 $\pm$ 0.118	108**
	600	8	100 (106g)	4.72 $\pm$ 0.107	116**
	900	7	100 (108g)	5.26 $\pm$ 0.139	130**
Clofibrate	150	9	100 (106g)	5.64 $\pm$ 0.106	139**
	300	6	100 (107g)	6.66 $\pm$ 0.149	164**

** significant $P < 0.01$ *Effect on serum cholesterol level*

Table 3 shows the inhibitory effect of alfibrate or clofibrate on the elevation of serum cholesterol, triglycerides, free fatty acids and phospholipids induced by the cholesterol diet. The elevation of serum cholesterol level in the control group from 80 mg% at the beginning up to 350 mg% at the end of the two experimental periods, was inhibited by 60–67% in the groups on alfibrate 150–900 mg/kg daily. The inhibition rate increased with the increasing dosage of alfibrate. The hypocholesterolemic effect of alfibrate appears to be comparable to that of clofibrate at these experimental dosages. Further investiga-

TABLE 3. Effect of alfibrate on serum lipid level in the hypercholesterolemic rats.

Drug administered (mg/kg daily)	Total cholesterol		Triglycerides		Free fatty acids		Phospholipids	
	mg%	% change	mg%	% change	μ mole/100 ml	% change	mg%	% change
None	349 $\pm$ 16.2 (10)		133.5 $\pm$ 5.44 (16)		96.5 $\pm$ 6.11 (16)		117 $\pm$ 3.2 (6)	
Alfibrate	150	140 $\pm$ 10.3 (9)**	107.7 $\pm$ 5.02 (8)**	–16	114.8 $\pm$ 6.06 (8)	–19	88 $\pm$ 4.8 (7)**	–25
	300	142 $\pm$ 14.4 (6)**	98.2 $\pm$ 3.25 (7)**	–23	93.6 $\pm$ 7.72 (7)	–3	80 $\pm$ 4.3 (6)**	–32
	600	122 $\pm$ 10.5 (8)**	100.8 $\pm$ 5.85 (8)**	–21	93.9 $\pm$ 4.87 (8)	–3	80 $\pm$ 2.6 (6)**	–32
	900	117 $\pm$ 9.5 (8)**	109.2 $\pm$ 5.85 (7)**	–14	89.5 $\pm$ 5.42 (7)	–7	85 $\pm$ 1.4 (6)**	–27
Clofibrate	150	129 $\pm$ 8.3 (9)**	89.4 $\pm$ 4.09 (9)**	–30	—	—	—	—
	300	129 $\pm$ 7.7 (6)**	84.7 $\pm$ 2.87 (6)**	–34	96.1 $\pm$ 6.87 (6)	0	78 $\pm$ 4.4 (8)**	–33

The drugs were orally administered for 2 weeks to rats on the cholesterol diet.

** significant $P < 0.01$

TABLE 4. Effect of alfibrate on serum β -lipoprotein level, and the ratios of serum free to esterified cholesterol and total cholesterol to phospholipids in the hypercholesterolemic rats.

Drug administered (mg/kg daily)	β -lipoprotein		Ratios of free/esterified cholesterol		Ratios of total cholesterol/phospholipids	
	mg% mean $\pm$ S.E.	% change	mean $\pm$ S.E.	% change	mean $\pm$ S.E.	% change
None	532 $\pm$ 26(7)		0.122 $\pm$ 0.003(7)		3.01 $\pm$ 0.17(6)	-42
Alfibrate 150					1.7 $\pm$ 0.18(7)**	-42
300	157 $\pm$ 19(8)**	-70	0.111 $\pm$ 0.003(8)*	-9	2.0 $\pm$ 0.23(6)**	-36
600	161 $\pm$ 18(8)**	-70	0.084 $\pm$ 0.002(8)**	-31	1.6 $\pm$ 0.15(6)**	-47
900	153 $\pm$ 15(7)**	-71	0.085 $\pm$ 0.010(7)**	-30	1.3 $\pm$ 0.14(6)**	-58
clofibrate 150					1.7 $\pm$ 0.23(8)**	-42

* significant $P < 0.05$ ** significant $P < 0.01$

tion with lower dosages, however, appears necessary. The data on liver weight in Table 2 and serum cholesterol level in Table 3 indicate that the degree of hepatomegaly does not appear to be relevant with the hypocholesterolemic effect.

Table 4 is a detailed analysis of serum cholesterol fraction. The increase of β -lipoprotein level was inhibited to a degree similar to that of serum cholesterol in alfibrate groups. Alfibrate significantly decreased the rate of free to esterified cholesterol level by increasing its metabolizable form, which would provide a favorable condition to metabolism of cholesterol. The values of total cholesterol to phospholipids were also lowered by 36–58% in proportion to the dosages of the drugs, thus the serum lipids were probably less atherosclerogenic.

Effect on serum triglycerides, free fatty acids and phospholipids

Alfibrate administration to rats on the cholesterol diet maintained the serum triglycerides level at approx. 98–109 mg% which would have reached 134 mg% without the treatment. The degree of inhibition was not in proportion to the dosage. Regarding the serum triglyceride level, clofibrate showed a greater hypolipidemic effect than alfibrate. In contrast to the results of normocholesterolemic rats in which serum triglycerides were more affected, the degree of serum cholesterol inhibition by the drugs was found to be 2–3 times greater than that of serum triglycerides in the hypercholesterolemic rats (Table 3). Serum free fatty acid level was not influenced, whereas the serum phospholipid level was significantly reduced to a slightly greater degree than that of triglycerides.

Effect on liver cholesterol concentration

The cholesterol diet resulted in an accumulation of cholesterol in the liver 10 times that of the normal and produced moderate fatty liver appearance. Alfibrate administration did not alter liver cholesterol level at either dosage but clofibrate resulted in a significant decrease at the higher dosage as shown in Table 5. Calculation for total quantity of liver cholesterol gives a different figure, that is, 15–19% increase in clofibrate groups due to a greater hepatomegaly with no change in alfibrate groups. Free cholesterol con-

TABLE 5. Effect of alfibrate on liver cholesterol concentrations in hypercholesterolemic rats.

Drug administered (mg/kg daily)	No. of rats	Total cholesterol		Free cholesterol		Ratios of free/esterified mean $\pm$ S.E. (% change)
		mg/g wet tissue mean $\pm$ S.E. (% change)	mg/liver	mg/g wet tissue mean $\pm$ S.E. (% change)		
None	10	54.1 $\pm$ 1.56	254.8 $\pm$ 8.03	5.01 $\pm$ 0.188		0.103 $\pm$ 0.0046
Alfibrate	150	57.0 $\pm$ 4.56 (+5)	248.8 $\pm$ 22.67 (-2.4)	3.91 $\pm$ 0.250** (-22)		0.077 $\pm$ 0.0059** (-25)
	300	54.1 $\pm$ 3.61 (0)	253.0 $\pm$ 29.72 (-0.7)	4.3 $\pm$ 0.310* (-14)		0.086 $\pm$ 0.0086 (-16)
Clofibrate	150	47.9 $\pm$ 2.66 (-11)	294.0 $\pm$ 16.75 (+15.4)	3.90 $\pm$ 0.259** (-22)		0.090 $\pm$ 0.0052 (-13)
	300	42.9 $\pm$ 1.84** (-21)	303.7 $\pm$ 15.56 (+19.2)	4.23 $\pm$ 0.116* (-16)		0.110 $\pm$ 0.0045 (+7)

Drugs were orally administered to rats on the cholesterol diet for 2 weeks.

* significant $P < 0.05$

** significant $P < 0.01$

TABLE 6. Effect of alfibrate on liver triglyceride, free fatty acid and phospholipid concentrations in hypercholesterolemic rats.

Drug administered (mg/kg daily)	No. of rats	Triglycerides		Free fatty acids		Phospholipids	
		mg/g wet tissue (mean $\pm$ S.E.)	% change	μ mole/g wet tissue (mean $\pm$ S.E.)	% change	mg/g wet tissue (mean $\pm$ S.E.)	% change
None	10	27.5 $\pm$ 2.86	0	115.7 $\pm$ 3.92	0	25.1 $\pm$ 0.49	0
Alfibrate	150	16.2 $\pm$ 2.38**	-41	61.8 $\pm$ 3.60**	-47	27.3 $\pm$ 0.76**	+9
	300	13.0 $\pm$ 1.14**	-53	74.0 $\pm$ 4.9*	-36	30.6 $\pm$ 1.02**	+22
Clofibrate	150	9.2 $\pm$ 0.68**	-66	51.5 $\pm$ 2.50**	-55	28.1 $\pm$ 0.41**	+12
	300	12.2 $\pm$ 2.36**	-56	50.5 $\pm$ 2.30**	-56	31.1 $\pm$ 0.62**	+24

Drugs were orally administered to rats on the cholesterol diet for 2 weeks.

* significant $P < 0.05$

** significant $P < 0.01$

centration in liver was significantly decreased by the treatment but only the alfibrate group at a lower dosage gave a significant decrease in the rate of free to esterified cholesterol.

Effects on liver triglycerides, free fatty acids and phospholipids

Table 6 shows the changes of triglyceride, free fatty acid and phospholipid concentration in liver by the drugs. In spite of a less degree of accumulation as compared to cholesterol, the concentration of triglycerides was also elevated to 27.5 mg/g tissue from 5 mg/g tissue in the control group when on the diet. Alfibrate inhibited the elevation of triglycerides by 41 and 53% respectively at the low and high dosages. Total quantity of triglycerides was calculated as follows; control: 129.2 mg, alfibrate 150 mg/kg: 75.2 mg, 300 mg/kg: 73.7 mg, clofibrate 150 mg/kg: 57.0 mg, 300 mg/kg: 85.7 mg. Even on the base of total quantity, alfibrate produced a 41.8 and 43.0% significant decrease of liver triglycerides at both low and high dosages, and clofibrate produced 55.9 and 33.7% decrease respectively. Free fatty acid concentration in liver was significantly decreased in

all experimental groups, whereas phospholipid concentration was increased significantly in proportion to the dosage. Total content of phospholipids in liver was control: 118.1 mg, alfibrate, low: 129.4 mg, high: 176.0 mg, clofibrate, low: 175.5 mg, high: 220.1 mg, which were equivalent to 9.6, 49.0, 48.6, 86.4% increase as percentage of the control. These results suggest that accumulation of phospholipids by the treatment is partly responsible for the hepatomegaly, however, an approx. 100 mg increase of phospholipids at the maximum appears to be too small an amount to explain the liver weight increase over 1 g.

Effect on total lipid concentration in various organs and adipose tissue

Since clofibrate is reported to stimulate lipogenesis and the related enzyme activities in rat adipose tissue in vitro (4), accumulation of lipids in peripheral organs and tissues is assumed to be one of mechanisms for the hypolipidemic action of alfibrate. The data in Table 7 shows the total lipid concentration in the liver, brain, kidneys and testes in the alfibrate group and control group with the result of no significant change. The weight of epididymal fat tissue was not significantly changed by alfibrate administration as shown in Table 8. Though further studies regarding the effect of alfibrate on adipose tissue are now in progress in this laboratory, the results herein suggest that divergence of serum lipids to various peripheral tissues does not appear to be a major mechanism in the lowering of serum lipids.

TABLE 7. Effect of alfibrate on total lipid concentrations in the various organs of hypercholesterolemic rats.

Alfibrate administered (mg/kg daily)	None		300	
	mg/g wet tissues mean $\pm$ S.E.		mg/g wet tissues mean $\pm$ S.E.	
Total lipids				
Liver	139 $\pm$ 3 (7)		133 $\pm$ 2 (8) N.S.	
Brain	76 $\pm$ 4 (7)		70 $\pm$ 3 (8) N.S.	
Kidneys	33 $\pm$ 1 (7)		36 $\pm$ 3 (8) N.S.	
Testes	27 $\pm$ 2 (7)		25 $\pm$ 2 (8) N.S.	
			% change	

N.S. $P < 0.1$

Numbers in parentheses indicate the numbers of rat employed.

TABLE 8. Ratios of epididymal fat tissue to body weight of hypercholesterolemic rats with and without alfibrate treatment.

Alfibrate administered (mg/kg daily)	0	300	600	900
Ratios of epididymal fat tissue to 100 g rat weight mean $\pm$ S.E. (No. of rats)	0.92 $\pm$ 0.05 (7)	0.79 $\pm$ 0.04 (8)	0.89 $\pm$ 0.05 (8)	0.95 $\pm$ 0.06 (7)
% change		-17	-7	-3

Effect on fatty acid distribution of serum and liver lipids

Fatty acid composition of serum and liver lipids was analyzed by gas chromatography to determine whether or not alfibrate influenced lipid metabolism qualitatively as well as quantitatively. Results with the serum and liver are shown in Tables 9, 10, respectively. Alfibrate administration produced a significant decrease of palmitic acid in serum and liver cholesterol esters, and had the general tendency to increase oleic acid in both serum and liver lipids and to decrease palmitoleic acid in liver cholesterol esters and triglycerides.

TABLE 9. Effect of alfibrate on fatty acid distribution of serum lipids in hypercholesterolemic rats.

Sources	Serum					
	Sterol esters		Triglycerides		Free fatty acids	
	0	300	0	300	0	300
Alfibrate administered (mg/kg daily)						
No. of rats	5	4	5	4	5	4
	Fatty acids (%) mean±S.E.					
C ₁₂	0.3±0.08	0.2±0.14	0.3±0.13	0.6±0.50	0.5±0.14	0.2±0.10
C ₁₄	1.4±0.25	1.1±0.38	1.9±0.49	3.8±2.23	1.8±0.12	1.7±0.14
C _{14:1}	0.2±0.10	0.6±0.22	0.5±0.27	4.8±2.13	0.5±0.04	0.4±0.03
C ₁₆	21.3±1.22	18.7±0.66	29.2±0.99	21.1±6.06	29.4±1.06	33.7±0.94*
C _{16:1}	12.9±0.79	10.1±1.65	8.8±0.81	12.9±1.66	5.7±0.19	6.5±0.36
C ₁₈	4.7±0.54	5.1±2.47	16.8±1.40	12.9±3.66	11.0±1.53	9.3±0.95
C _{18:1}	43.5±1.40	50.0±3.73	32.1±1.68	35.0±4.26	33.3±2.61	37.3±1.88
C _{18:2}	14.4±0.93	13.3±0.74	10.2±0.71	8.9±2.22	15.9±1.49	11.7±1.43
C _{20:2}	1.4±0.75	1.3±1.58	—	—	2.3±1.80	0.5±0.35

* significant $P<0.05$

TABLE 10. Effect of alfibrate on fatty acid distribution of the liver lipids in the hypercholesterolemic rats.

Sources	Liver			
	Sterol esters		Triglycerides	
	0	300	0	300
Alfibrate administered (mg/kg daily)				
No. of rats	4	4	4	4
	Fatty acid (%) mean±S.E.			
C ₁₄	0.6±0.04	1.0±0.25	1.1±0.12	0.9±0.17
C _{14:1}	—	—	0.1±0.03	0.2±0.23
C ₁₆	24.6±0.44	21.0±0.89*	33.9±0.92	34.0±1.25
C _{16:1}	13.8±0.36	10.9±1.27	5.7±0.40	3.4±0.26*
C ₁₈	1.8±0.17	2.9±0.35*	1.9±0.07	3.3±0.53
C _{18:1}	51.1±0.74	56.0±3.63	47.0±0.89	43.1±1.88
C _{18:2}	7.7±0.60	9.6±2.20	10.3±0.74	13.5±1.92

* significant $P<0.05$

DISCUSSION

Regardless of the remarkable reduction in serum cholesterol level in hypercholesterolemic rats, alfibrate produced no change on liver cholesterol concentration and the only significant decrease was observed in 300 mg/kg clofibrate group probably due to dilution by hepatomegaly. Morris and Chaikoff (5) reported that when there was only 0.05% cholesterol in the diet, 70–80% of cholesterol was synthesized within the body whereas on a diet containing 2% cholesterol the endogenous production fell to 10–30%. In our experimental conditions with 1% cholesterol diet, the hepatic cholesterol synthesis was probably much reduced. Four major mechanisms considered to act in lowering serum cholesterol are: 1) Inhibition of hepatic cholesterol synthesis, 2) Inhibition of hepatic cholesterol transport, 3) Stimulation of cholesterol catabolism to bile acids 4) Inhibition of intestinal absorption of cholesterol, and 2), 3) or 4) should be given special consideration herein as the sites affected by alfibrate in the experiments. Significant reduction of cholesterol in serum with no change in the liver, and a concomitant decrease of triglycerides in serum and liver by the drug treatment, provide strong evidence that alfibrate primarily inhibits biosynthesis of triglycerides and secondarily interferes with the transport of cholesterol from liver as lipoproteins due to the shortage of a constituent, triglycerides. The raised cholesterol level in liver thus occurring in the treated groups is assumed to be approximately reduced to the levels in the control groups through the inhibition of cholesterol biosynthesis or the enhancement of the excretion as sterols and bile acids by alfibrate or clofibrate.

The hepatic enlargement by clofibrate is known to be associated with accumulation of lysosome like organelles within cytoplasm (6). The enhancement of mitochondrial glycerophosphate dehydrogenase activity with a concomitant decrease of α -glycerophosphate level in cytoplasm, thereby reducing the availability of α -glycerophosphate for triglyceride synthesis (7) is a most reasonable mechanism for the inhibition of triglyceride synthesis by clofibrate. In fact, it was observed in this laboratory that there was a 30% and 33% decrease of α -glycerophosphate in the livers of alfibrate and clofibrate treated rats respectively. The finding that a greater decreased rate in serum and liver triglycerides was observed in clofibrate group with a greater hepatomegaly than in alfibrate, together with the reports mentioned above, may relate the hypotriglyceridemic effect to the liver enlargement.

Alfibrate affected neither the total lipid concentration in the brain, liver, kidneys and testes nor the weight of epididymal fat tissue. Although the effect was not manifested on the base of weight alfibrate may modify the lipoprotein lipase activity, hormone sensitive lipase activity and also the activities of some related enzymes in adipose tissue.

Analysis of fatty acid distribution in serum and liver lipids showed that in general, oleic acid tended to increase with alfibrate treatment, this finding coinciding with the result in humans. The physiological significance, however, remains virtually unknown.

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