

Possible Involvement of Hepatic Phosphatidate Phosphohydrolase in the Mechanisms of Actions of Certain Antilipemic Drugs in Rats

Bahram Haghghi*, Mani Kharazizadeh and Mohammad Ali Attar

Department of Clinical Biochemistry, School of Pharmacy, Medical Sciences University of Esfahan, Esfahan, Iran.

Abstract

The effects of therapeutic doses of dillsun, garsin, antum and statins on rat liver cytosolic phosphatidate phosphohydrolase (PAP) activity, a key enzyme in triacylglycerol synthesis, and on serum and liver lipids were examined. Lovastatin and simvastatin both stimulated the enzyme activity by 29% and 43%, respectively. The stimulatory effects were dose-dependent and accompanied by the decline in triacylglycerol and cholesterol concentrations of serum (20-29%) and liver (12-29%) suggesting a possible feed-back mechanism for PAP control. Dillsun (0.5-2 ml/day) did not affect PAP activity and liver lipids but lowered serum triacylglycerol (27%) and cholesterol (20%) concentrations at a dose of 2 ml/day. Antum administration also showed similar pattern as dillsun except that it caused a decrease in the liver lipids as well. Garsin (250-1000 mg/Kg) inhibited PAP activity (14-22%) with simultaneous decrease in the liver and serum lipids; maximum decrease in serum triacylglycerol and cholesterol were 14% and 21%, respectively. The data demonstrated that the antilipemic drugs tested exert their effects, at least in part, through alteration of hepatic PAP activity.

Keywords: Phosphatidate phosphohydrolase; Lovastatin; Simvastatin; Dillsun; Antum; Garsin.

Introduction

Phosphatidate phosphohydrolase (PAP, EC 3.1.3.4.) catalyzes desphosphorylation of phosphatidic acid. Two distinct forms of PAP have been identified in rat liver (1). The metabolic form is located in cytosol and microsomal fraction, requires Mg^{2+} and is a regulatory enzyme in triacylglycerol and phospholipids biosynthesis (2, 3). The second form of PAP present in the plasma membrane, does not require Mg^{2+} and is involved in signal transduction (4). The metabolic form of PAP in the cytosol is in its active form and can be

translocated to the microsomal membrane, where triacylglycerol synthesis occurs, by certain regulatory factors such as fatty acids and acyl-CoA, in liver (5-6), heart (7) and adipose tissue (8). The activity of soluble PAP may also be regulated by certain hormones such as steroid, glucagon (9) and epinephrine (10).

The rate at which triacylglycerol is secreted into the blood stream in the form of VLDL, and the rate of its clearance from the blood are factors that determine lipid levels in the serum. Several drugs have been widely used as antilipemic agents (11). The mechanisms by which these compounds affect serum lipid levels, however, are still a contradictory subject. It has been reported that the hypolipidemic effects of some drugs such as nicotinic acid, clofibrate and

* Corresponding author:

E-mail: bahram_haghghi5@pharm.mui.ac.ir

gemfibrozil are, at least in part, resulted from the inhibition of hepatic PAP activity in rat (12). Among statins HMG-CoA reductase inhibitors (13, 14), lovastatin and pravastatin have different effects on PAP activity (15) contradictory to those reported for other antilipidemic drugs (12).

Several medicinal plants such as garlic (*Allium sativum*) (16) and anethum (*anethum graveolens*) (17, 18) have been used as antilipidemic drugs. The mechanisms of actions of these drugs, however, are not clarified yet. Three antilipidemic drugs whose constituents are anethum (Antum and Dillsun) and garlic (Garsin) are produced by the Iranian pharmaceutical industries, but the detailed mechanisms of their actions have not been studied. Antum is a combination of fumaria, anethum and chichorium which is distributed by Iran Daruk Co. in the forms of tablets or granules. Dillsun is a 2% essence of anethum presented by Barij Esans Co. as drops. Garsin is a powder of garlic as tablets produced by Barij Esans Co.

In the present study the effects of therapeutic doses of dillsun, garsin, antum, lovastatin and simvastatin on the metabolic form of PAP activity and on the serum and liver lipids of rats were investigated.

Experimental

Materials

Phosphatidic acid (sodium salt), dithiothrietol, EDTA and bovine serum albumin were purchased from Sigma Co. (U.S.A). Lovastatin was from Tehran Chimie (Iran) and simvastatin obtained from Shahre Darou Co. (Iran). Dillsun, antum and garsin were received from Barij Esans Co. (Iran), Iran Darouk Co. (Iran) and Gol Darou Co. (Iran), respectively. All other reagents were of the highest grade available.

Animals

Male Wistar rats (from Pasteur Institute, Iran) were fed and maintained as described before (19) except that their food contained 2% cholesterol and 0.5% cholic acid to induce hyperlipidemia (20). The rats received simvastatin or lovastatin at daily doses of 20 and 40 mg/Kg through feeding tube for 3 days. Dillsun and garsin were

also given daily at various doses (see legends) by the feeding tube for 9 and 30 days, respectively. Antum powder was added to the food (10%) and the animals fed, ad libitum, for 15 days. Control animals for each drug were treated the same way except that the drug was omitted from the solvent or food. The rats were sacrificed by decapitation, the blood samples collected and each liver was homogenized.

Preparation of liver homogenate

The liver of each rat was perfused with isotonic NaCl to remove the blood using single passage system and homogenized as described previously (12) except that the homogenizing solution was 50 mM Tris-HCl buffer pH 7.5 containing 1 mM EDTA and 0.225 M sucrose. The homogenate was centrifuged at 12000 ×g for 30 min and the supernatant kept for the enzyme assay.

Assay of PAP activity

The activity of PAP was measured as reported before (12).

Other methods

Protein concentration was determined by the method of Lowry et. al (21). Cholesterol and triacylglycerol in the serum were measured by commercial enzymatic kits (Darman kave, Iran). The lipids in the liver samples were first extracted in chloroform :methanol (2:1) according to Norman's method (22) and then measured as serum samples. Student t-test was used for statistical analysis.

Results

The changes in hepatic PAP activity and in serum and liver lipids induced by injection of rats with lovastatin and simvastatin are shown in Table 1. Both lovastatin and simvastatin stimulated hepatic PAP activity. The increase in the enzyme activity was concentration dependent being 29% and 43% at (40 mg/Kg) dose for lovastatin and simvastatin, respectively. The stimulatory effects were accompanied by the decline in triacylglycerol and cholesterol concentrations of serum and liver. Lovastatin (40 mg/Kg) decreased triacylglycerol of serum

Table 1. The effects of lovastatin and simvastatin on hepatic PAP activity and on serum and liver lipids.

Drug (mg/kg)	PAP activity (nmole Pi/min/mg protein)	Triacylglycerol		Cholesterol	
		Serum (mg/dl)	Liver (mg/g of liver)	Serum (mg/dl)	Liver (mg/g of liver)
Lovastatin:					
None(control)	3.88±3.0	82.4±5.6	5.2±0.5	84.4±5.8	6.7±0.5
20	4.8±0.64*	61.9±4.8*	4.4±0.5*	58.7±5.2*	5.2±0.5*
40	5.27±0.45*	58.8±6.0*	4.2±0.4*	53.3±5.5*	4.9±0.4*
Simvastatin:					
None(control)	4.32±0.42	75.1±4.1	7.5±0.8	80.4±5.0	3.9±0.4
20	6.29±0.61*	60.6±2.9*	6.8±0.8*	58.0±2.5*	3.3±0.3*
40	7.16±0.76*	54±2.1*	6.6±0.7*	49.8±1.7*	3.2±0.2*

Rats were given lovastatin or simvastatin at daily doses of 20 and 40 mg/Kg through feeding tube for 3 days. Control rats received the solvent. PAP activity and lipid concentrations were measured as described in Methods. The values represent mean±SD of 5 rats.

* Significantly different from control ($p < 0.05$).

(20%) and liver (29%), whereas the decrease induced by simvastatin in the same tissues were 29% and 12%, respectively. The decrease in cholesterol of rats receiving lovastatin was 37% in serum and 28% in liver. Similar patterns of cholesterol changes were observed for simvastatin.

Table 2 demonstrates the effects of dillsun, antum and garsin on the hepatic PAP activity and the lipid concentrations of serum and liver. Although dillsun (0.5–2 ml/day) did not affect PAP activity and liver lipids significantly but lowered serum triacylglycerol (22%) and cholesterol (20%) at 2 ml/day dose. Antum also did not significantly change PAP activity but decreased serum triacylglycerol (12%) and cholesterol (15%). Antum also lowered the liver lipid content. Garsin inhibited PAP activity at 500 mg/day (14%) and 100 mg/kg (22%) with simultaneous decrease in the serum and liver lipids (Table 2). Maximum decreases for serum triacylglycerol and cholesterol levels were 14% and 21%, respectively.

Discussion

The finding that certain antilipidemic drugs such as nicotinic acid, clofibrate or gemfibrozil exert their effects, at least partly, through affecting hepatic PAP activity (12) led to the assumption that the activity of this enzyme might also be altered by other drugs such as those investigated

in this study. Statins are known as HMG-CoA reductase inhibitors (23). In the present study both lovastatin and simvastatin decreased triacylglycerol and cholesterol concentrations in both serum and liver. This may be explained by reduced synthesis of these lipids in the liver and/or decreasing their secretion into the blood as VLDL. Simvastatin also increases LDL receptors resulting in removal of LDL and its precursors from the serum. It seemed likely that these compounds also inhibit PAP activity which is a key enzyme in triacylglycerol synthesis (1), but surprisingly PAP activity was slightly elevated with simultaneous decrease in triacylglycerol levels in both liver and serum. This may be explained by several assumptions: 1) Previous reports have shown that the activity of HMG-CoA reductase upon statin treatment is primarily increased (15, 25). These studies have also shown that both HMG-CoA reductase and PAP exhibit a co-alteration upon administration of certain drugs such as cholesteramine (15). Several explanations may be proposed for these findings.

2) A correlation between PAP and HMG-CoA reductase activities have been previously reported (15). The increased HMG-CoA reductase, therefore, may stimulate PAP activity through a compensatory mechanism.

3) A feed back control may increase PAP activity after reduction of serum triacylglycerol concentration.

Table 2. The effects of Dillsun, Antum and Garsin on hepatic PAP activity and on serum and liver lipids.

Drug Doses	PAP activity (nmolePi/min/mg protein)	Triacylglycerol		Cholesterol	
		Serum (mg/dl)	Liver (mg/g of liver)	Serum (mg/dl)	Liver (mg/g of liver)
Dillsun (ml /day):					
None (control)	3.71±0.35	101.3±5.9	5.7±0.4	112±4.8	7.6±0.5
0.5	3.58±0.67	95.7±5.0	5.5±0.4	103±3.2	7.2±0.4
1	3.43±0.48	88.5±5.9	5.3±0.4	97±4.5	6.9±0.3
2	3.23±0.58	78.9±6.4	4.9±0.4	90±4.4*	6.6±0.5
Antum:					
None (control)	6.74±0.8	112±4.8	8.9±1	121±5.1	5.7±0.7
10% in food	6.14±0.52	98±3.4*	8.0±0.9*	103±3.9*	4.9±0.6
Garsin (mg/Kg):					
None (control)	6.31±0.64	193±11.8	8.5±1	208±13.2	5.9±0.7
250	5.69±0.47 *	187±10.4	8.1±0.9	190±11.4	5.6±0.7
500	5.40±0.44 *	175±9.5*	7.8±0.8*	171±8.9*	5.2±0.6*
1000	4.94±0.32 *	166±7.9*	7.4±0.8*	164±7.8*	4.8±0.5*

Rats received dillsun or garsin at daily indicated doses through feeding tube for 9 and 30 days, respectively. Rats receiving antum were fed with food containing 10% antum powder, ad libitum, for 15 days. Control animals for each drug treated the same way but omitting the drug. PAP activity and lipid concentrations were measured as described in Methods. The values represent mean ± SD of 5 rats.

* Significantly different from control (p<0.05)

4) Statins may affect fatty acid and acyl-CoA metabolism which in turn stimulates translocation of PAP from cytosol to endoplasmic reticulum where its activity increases (6).

5) Statins exert some of their effects through β -adrenoceptor activation resulting in an increase in cAMP concentration (14) which in turn may stimulate PAP activity in the experimental conditions as it does for glucose 6-phosphate dehydrogenase in isolated human hepatocytes (26).

The antilipidemic effect of garsin in both serum and liver (Table 2) may be attributed to the decrease in hepatic lipid synthesis and VLDL secretion into the blood which is supported by its inhibitory effect on PAP activity, a key enzyme in triacylglycerol synthesis. Garsin also contains flavonoid compounds (27) and in animals fed with this compounds serum triacylglycerol has declined (28). The later report suggested that garlic (the effective portion of garsin) may prevent lipid absorption in the intestine and increase its consumption in the muscles resulting in a decrease in serum lipids. Another compound present in garlic is allicin which in turn lowers serum cholesterol and

triacylglycerol concentrations through inhibition of the activities of HMG-CoA reductase (28, 29) and PAP (Table 2) in the liver.

The mechanism by which antum lowers cholesterol and triacylglycerol is not well understood. Triacylglycerol synthesis is not involved in this mechanism since PAP activity was not affected. The major components present in antum are anethum (68%), fumaria (5%) and chichoriom (5%). The effects of antum, therefore, may be attributed primarily to anethum. Previous studies have revealed that anethum lowers total cholesterol, LDL-cholesterol and increases HDL-cholesterol (18). The major components of the essence of anethum such as carvon, limonen or α -phellandrene (30) may be responsible for the anethum effects possibly through affecting the key enzymes such as HMG-CoA reductase or acyl-CoA carboxylase. Anethum components may also increase LDL receptors in the liver stimulating cholesterol clearance from the blood.

Dillsun showed similar results to antum on serum lipids, only at 2 ml/day dose, but did not change liver lipids. Since the major component of dillsun is anethum it is likely that the differences

observed are associated with the drug doses applied. Nevertheless, other components in antum such as fumaria and chichorium may be also accounted for the difference between these two drugs.

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