EFFECT OF MEDROXY PROGESTERONE ACETATE ON HEPATIC LIPID PROFILE OF FEMALE RATS UNDER VARIOUS STATES OF NUTRITION

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Summary : The effect of medroxyprogesterone acetate (MPA) treatment on hepatic lipid profile was studied in female rats kept on protein-deficient diet, on normal restricted diet and on normal, *ad libitum* diet. A significant decline in total and free cholesterol levels was observed in rats kept on protein-deficient diet and on normal, restricted diet. However, protein-deficient animals exhibited a significant rise in the liver triglyceride level. In rats on normal, *ad libitum* diet only, MPA treatment resulted in elevated levels of triglycerides and increased esterification of cholesterol. This was mostly due to increased incorporation of acetate into esterified cholesterol and triglyceride as evident from studies using the abelled precursor. Total phospholipid content was found to be unaffected by MPA in all the groups suggesting that the drug and dietary protein level have no effect on hepatic phosphloipid content.

Key words : medroxyprogesterone acetate, protein deficiency, hepatic lipids, acetate-2-14C-incorporation

INTRODUCTION

The effects of medroxyprogesterone acetate (MPA) on serum and hepatic lipids have been recently studied in rats by Dutta *et al.* (3). These workers reported a significant elevation in blood cholesterol and triglyceride levels at a dose of 2 *mg*/100 *g*. These changes may be either due to the alteration in very low-density lipoproteins (VLDL) and high density lipoproteins (HDL), which play an important role in the transport of lipids (2, 11), or due to increased synthesis of individual lipids.

The present study was designed to investigate the effect of MPA in the liver lipids of rats fed nomral *ad libitum* diet and in rats in protein deficient state. Furthermore, incorporation of labelled acetate-2-^{14}C in neutral and phospholipids was studied in rats fed normal *ad libitum* diet.

MATERIAL AND METHODS

Female albino rats of Wistar strain (150-175 g) were used. One group (n=20) was fed a synthetic diet (composed of casein (20%), corn starch (40%), dextrose (30%), refined peanut oil (5%), salt mixture (USP XIV) (1%), cholinechloride (0.2%) and vitamin mixture (1%)) *ad libitum*. Another group (n=20) was given protein-deficient diet (composition as above, but containing 8% casein only, the diet being made isocaloric with starch). The consumption of the diet by this group was measured daily and a third group of rats (n=20) was given the same bulk of diet as consumed by protein-deficient animals (normal restricted diet group). The body weights of the animals were recorded weekly.

The animals on each dietary schedule were further divided into two subgroups (control and experimental) after 15 days. The control animals were injected im with 0.1 *ml* vehicle only (polysorbate 80, 0.237 *mg*; methylparaben, 0.1349 *mg*; propylparaben, 0.0147 *mg*; polyethylene glycol 4000, 2.852 *mg* and sodium chloride, 0.8567 *mg*/0.1 *ml*) every week. The experimental animals were given im injection of 3.5 *mg*/100 *g* of medroxyprogesterone acetate (MPA; Depo provera*, Upjohn, Belgium) per week. A total of four injections were administered and animals sacrificed 5-6 days after the last injection.

Livers were removed, blotted and weighed; 1.0 to 1.5 *g* of the tissue was taken for the extraction of lipids. It was homogenised in 20 volumes of 2:1 chloroform-methanol mixture in mortar containing acid-washed sand and the lipids were extracted according to the method of Folch *et al.* (4).

The individual phospholipids in the lipid extract were separated by thin layer chromatography (TLC) using a solvent system comprising of chloroform-methanol-ammonia (65 : 25 : 4, v/v). The chromatogram was developed in iodine vapours, the spots eluted and phospholipid phosphorus estimated according to Bartlett (1).

The neutral lipids were also separated by TLC. The solvent system consisted of petroleum ether-solvent ether-glacial acetic acid (90:10:1, v/v). The chromatogram was developed in iodine vapours. The spots were eluted, and the free and esterified cholesterol were estimated by the procedure of Zak (12) and triglycerides by the method of Gottfried and Rosenberg (6).

For studying the incorporation of labelled acetate into the liver lipids, acetate-2-^{14}C (specific activity, 46.15 *mCi/mmole*) was given ip (10 μ*Ci*/100 g) and the animals were

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sacrificed after 2 hours. The individual spots of the lipids obtained by TLC were scraped and put into the scintillation vials containing 10 *ml* of toluene based scintillation fluid. Radioactivity was counted with a Rackbeta Liquid Scientillation Counter.

Statistical analysis : Student's "t" test was used to evaluate differences between the groups.

RESULTS AND DISCUSSION

In the present study, total serum protein level and albumin/globulin (A/G) ratio were used as indices for ascertaining the induced protein deficiency. A significant decrease ($P<0.01$) in the total serum protein content (mean values; control, 7.3 ± 0.80 *g*%; protein-deficient, 4.9 0.66g%) and A/G ratio (mean values, normal *ad libitum* diet 0.92 ± 0.08; protein-deficient diet, 0.75 ± 0.065) was observed in protein-deficient animals.

The effect of diet and MPA treatment on liver cholesterol and triglyceride content in rats on different diet schedules is presented in Table I. A significant reduction in the

TABLE I : Effect of medroxyprogesterone acetate (MPA) on cholesterol, triglyceride and individual phospholipid contents of the liver (*mg/g* tissue) in female rats.

Diet	*Subgroup*	*Total cholesterol*	*Free cholesterol content*	*Triglyceride*	*Sphingomyelin*	*Phosphatidyl ethanolamine*
Normal *ad libitum* diet	Control	4.262±0.438	3.661±0.329	6.238±0.736	1.475±0.461 (10.40)	3.989±0.897 (28.13)
	MPA	4.782±0.425	3.852±0.308	9.421±1.040^{**a}	2.626±0.818^{*a} (22.74)	2.065±0.795^{*a} (17.89)
Normal restricted diet	Control	3.523±0.394^{*a}	2.977±0.212^{*a}	7.870±1.462	2.012±0.529 (15.55)	2.548±0.613 (19.69)
	MPA	3.744±0.391	3.124±0.271	10.668.1±561^{*c}	2.286±0.583 (17.93)	2.089±0.512 (16.38)
Protein-deficient diet	Control	3.431±0.380^{*a}	2.966±0.242^{*a}	9.510±1.758^{*a}	3.156±0.875^{*a} (25.23)	1.142±0.327$^{***a, **b}$ (9.13)
	MPA	2.854±0.302^{*b}	2.398±0.208^{**b}	8.382±2.124	2.972±0.814 (26.11)	0.953±0.282 (8.37)

Values are mean (±S.D.) of 4 observations.
Values within parentheses are percent of total phospholipids per g tissue.
Value differs significantly ($^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$) from group fed normal diet *ad libitum* (a), protein-deficient diet (b) or normal restricted diet (c).

total and free cholesterol was observed in rats on protein-deficient diet and in rats on normal, restricted diet. Administration of MPA significantly increased ($P<0.01$) esterification of cholesterol (from 0.45 ± 0.07 to 0.75 ± 0.1 *mg/g* of liver tissue) in the rats on normal, *ad libitum* diet. This also produced significant reduction in the total and free cholesterol in rats on protein-deficient diet, though not in the two other groups. Triglyceride levels were found to be significantly elevated in control rats on protein-deficient diets compared to control rats on normal, *ad libitum* diet. The triglyceride levels also increased significanltly in rats on normal, *ad libitum* diet and in rats on normal, restricted diet following the drug treatment. However, the administration of the drug did not cause any significant change in the triglyceride contents in the protein deficient animals.

Phospholipid content of liver of rats on normal, *ad libitum* diet was 14.18 ± 3.15 *mg/g*. This was not significantly changed by change in diet in other two groups or by MPA-administration.

The effect of MPA treatment on individual phospholipids (sphingomyelin and phosphatidylethanolamine) in various groups is presented in Table I. Protein-deficient animals showed a significant rise in the sphingomyelin content but a significant fall in the phosphatidylethanolamine fraction when compared to the other two groups. The MPA treatment caused significant changes in the individual fractions of phospholipids in the normal, *ad libitum* group only. There was a significant rise in the contents of phosphatidylinositol plus phosphatidylserine fraction and sphingomyelin but a significant decrease in the phosphatidylcholine and phosphatidylethanolamine fractions was observed as a result of drug treatment. The contents of individual phospholipid fractions in the other two groups (normal, diet restricted and protein-deficient) were not altered following MPA-administration.

A significant increase in the incorporation of the labelled precursor (acetate-2-^{14}C) into esterified cholesterol and triglyceride was noted (Table II) after the MPA administration in the rats on normal, *ad libitum* diet. This experiment explains the increased esterified cholesterol and triglyceride level noted in the group of MPA-treated rats on normal, *ad libitum* diet.

A significant increase in the incorporation of labelled acetate into phosphatidylinositol plus phosphatidylserine and the sphingmyelin fractions was seen in the drug-treated rats on normal, *ad libitum* diet (Table III); however, the incorporation study revealed a significant decline in the incorproation of the precursor in the phosphatidylcholine and phosphatidyl-ethanolamine in this group.

TABLE II : Effect of medroxyprogesterone acetate (MPA) treatment on acetate-2-14C incorporation into neutral lipids in rat liver.

Lipid fraction	cpm/g tissue		cpm/mg individual lipid	
	Control	MPA-treated	Control	MPA-treated
Triglycerides	233.33±18.84	440.67±41.96***	37.40±3.21	46.77±4.27**
Free cholesterol	2983.33±257.27	3469.67±350.59	814.89±59.18	900.74±56.70
Esterified cholesterol	261.33±19.37	505.33±45.20***	582.03±48.94	668.43±50.97*

Values are mean (±S.D.) of 4 observations.
Value significantly differs from control; *P<0.05; **P<0.01; ***P<0.001.

TABLE III : Effect of medroxyprogesterone acetate (MPA) treatment on acetate-2-14C incorporation into phospholipids in rat liver.

	cpm/g tissue	
	Control	MP-Atreated
Total phospholipid	7566.67±995.29	6141.00± 700.67
Phosphatidylinositol + Phosphatidylserine+ Lysophosphatyl choline	485.00±38.81	1033.33±130.41***
Sphingomyelin+Lysophosphatiydl ethanolamine	543.33±50.21	753.33±68.29**
Phosphatidylcholine	1500.00±166.82	960.00±81.17***
Phosphatidylethanolamine	3650.00±390.16	2216.67±190.18***

Values are mean (±S.D.) of 4 observations.
Value significantly differs from control; *P<0.05; **P<0.01; ***P<0.001.

Fat accumulation in the liver may either be due to secretion of hepatic triglycerides at a rate below that of their synthesis, or alternatively, due to increased production of triglycerides at a rate which surpasses the ability of liver to secrete them (7,10). Most triglycerides leave the liver in the form of very low-density lipoprotein (VLDL) particles that contain in addition to triglycerides, varying amounts of cholesterol and its ester, phospholipids and protein.

It has been reported that significant change did not occur in VLDL in well nourished rats treated with MPA and it was pointed out that an increase observed in hepatic triglycerides was not due to impairment of transportation (3).

The results obtained in the present study indicate that the changes in the liver triglycerides due to MPA-treatment may probably be due to increased triglyceride production.

MPA has long been known as a steroid having properties similar to corticosteroid hormones (5). Hill *et al.* (8,9) have demonstrated that cortisone treatment showed two fold increase in the non-phospholipid hepatic glycerols, without any change in total hepatic cholesterol or phospholipid contents in rats. Our study indicates a striking similarity between effects of MPA in the well nourished rats and effects reported with cortisone (8,9).

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