THE EFFECT OF TETRACYCLINE ON SYNTHESIS OF FATTY ACID AND CHOLESTEROL IN THE LIVER OF CONTROL AND EXPERIMENTAL RATS

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(Received for publication October 19, 1970)

Tetracycline-induced fatty metamorphosis of the liver[1] of human and experimental animals has already been reported. EINHEBER *et al.*[2] demonstrated that there is increased accumulation of glyceride in the liver of mice after administration of chlortetracycline. In tetracycline-treated rat the secretion of triglyceride by the liver is impaired[3] and the stimulation of triglyceride synthesis observed in liver of drug-treated animals is due to preferential utilization of free fatty acids.

In the liver, triglyceride synthesis is believed to occur in microsomal tissue and soluble cytoplasm[4]. The fatty acid portion of the molecule is derived either from plasma or endogenously formed free fatty acids within the liver. We have demonstrated[5] that in experimental animal, tetracycline enhances the release of free fatty acids from adipose tissue, resulting in, elevation of plasma free fatty acid concentration. The present study describes the effect of antibiotic on fatty acid and cholesterol biogenesis in the liver slices of tetracycline-treated rats when the drug was administered at a therapeutic level.

Materials and Methods

Tetracycline hydrochloride was obtained from Standard Pharmaceuticals Ltd., Calcutta. Adenosine triphosphate (ATP), glutathione (GSH) and diphosphopyridine nucleotide (NADH) were obtained from Sigma Chemical Co., U. S. A. 2-C^{14}-Acetate and 2-C^{14}-malonate of Radiochemical Centre, Amersham, England was used in this study.

Male albino rats weighing 100~150 g were kept on stock ration. Tetracycline hydrochloride in aqueous solution was injected intramuscularly daily, for a period of 4 weeks. Maximum and minimum dose levels of therapeutic recommendation of the drug were used during the course of investigations. The animals were sacrificed three hours after the last dose of the drug. The livers were excised, weighed and transferred to a beaker containing ice-cold phosphate buffer (pH 7.4). Liver slices of approximately 0.5 mm thickness were obtained by using Arthur Thomas hand microtome. Approximately 500~600 mg of slices were used for each incubation. The incubation was carried out at 37°C for 3 hours according to MUKHERJEE and ALFIN-SLATER[6]. At the end of incubation the medium was decanted, the slices were washed thrice with distilled water, hydrolysed with alcoholic potassium hydroxide for 30 minutes and finally the lipid was extracted following the procedure of HIRSCH *et al.*[7]. An aliquot of chloroform extract was heated on a water bath to remove the solvent. The residue was dissolved in 5 ml of alcohol - acetone (1 : 1) mixture and the cholesterol was precipitated as digitonide after adding 5 mg of carrier-cholesterol. It was allowed to stand overnight, centrifuged and the clear solution was saved. The precipitate was washed twice with alcohol - acetone mixture and finally with diethyl ether twice to remove the adhering fatty acids from cholesterol-digitonide. The cholesterol-digitonide precipitate was placed in the aluminum planchet from alcoholic suspension for measurement of radioactivity.

Total wash was mixed with previous solution saved for isolation of fatty acids. The solution was evaporated to dryness and extracted with 5 ml of petroleum ether (b. p. 40~60°C) twice. The solvent was evaporated and the fatty acid was dissolved in 5 ml chloroform. One ml of chloroform extract was used for plancheting and after removing the solvent, the radioactivity was measured with the Tracer Laboratory Model SA-SC ISA Super Scalar gas flow counter.

Results and Discussion

The results of incorporation of 2-C^{14}-ace-

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Table 1. Incorporation of 2-C^{14}-acetate into fatty acids and cholesterol in liver slices of control and tetracycline-treated rats

	Dose of tetracycline (mg/100 g)	Acetate radioactivity incorporation into	
		Fatty acid (cpm/mg of protein)	Cholesterol (cpm/mg of protein)
Control (7)	—	67±5.8	23±0.83
Tetracycline treated (7)	1.5	138±33	11±6.8
Tetracycline treated (8)	3.5	9±1.85	5±1.1

Tetracycline treatment was continued for 4 weeks. Figures in parenthesis represent number of animals.

Each incubation flask contains: Approximately 0.5 g liver slices, 1.5 μmoles ATP, 7.5 μmoles $MgCl_2$, 10 μmoles GSH, 40 μmoles niacinamide, 20 μmoles phosphate buffer (pH 7.4), 1 μmole KCl, 1 μmole NaDH and 1 μmole 2-C^{14}-acetate (cpm-37,000) in a total volume of 2.0 ml.

Table 2. Effect of tetracycline administration on the conversion of 2-C^{14}-acetate and 2-C^{14}-malonate into fatty acids in the rat liver slices

	Incorporation into long-chain fatty acids	
	2-C^{14}-Acetate (cpm/mg protein)	2-C^{14}-Malonate (cpm/mg protein)
Control (6)	422 ± 55	2686 ± 227
Tetracycline (6) (3.5 mg/100 g)	144 ± 12	2630 ± 310

Drug was administered for 7 days.

Figures in parenthesis represent number of animals. Duplicate incubation was conducted with slices from liver of each animal.

Each incubation flask contained: Approximately 0.5 g liver slices, 1 μmole 2-C^{14}-acetate (3.7×10^5 cpm) or 1 μmoles 2-C^{14}-malonate (3.7×10^4) and all the reagents used in experiment described in Table 1.

tate into higher fatty acids and cholesterol in the liver slices of control and tetracycline-treated rats are presented in Table 1. The effects of tetracycline on fatty acid and cholesterol biogenesis in rats were studied after administration of drug at the dose levels of two different regimes of therapeutic recommendation. Animals receiving daily 1.5 mg of tetracycline hydrochloride for 100 g of body weight showed a pronounced stimulation of fatty acid synthesis from 2-C^{14}-acetate. BELL and CONIGLIO[8] also demonstrated similar stimulation of fatty acid biosynthesis in tetracycline-treated rats, however, in their experiment the doses of antibiotic administered was significantly below the therapeutic level. When the dose level of antibiotic was increased to 3.5 mg per 100 g of body weight of the animals, the incorporation of 2-C^{14}-acetate into long chain fatty acids was significantly reduced. The biogenesis of cholesterol, however, is lowered by tetracycline administration irrespective of the doses, the effect being more pronounced when the dose of the drug administered was higher.

The rates of biogenesis of fatty acids bear little resemblance to the actual lipid picture[5] of the liver under the influence of antibiotic. While markedly elevated levels of the lipids are observed in rats receiving 3.5 g of tetracycline per 100 g of the body weight, the rate of fatty acid synthesis is considerably lowered. The pronounced hepatotoxic effect of tetracycline seems to be responsible for such an inhibition. Stimulation of fatty acid synthesis is observed in rats receiving 1.5 mg of tetracycline, but accumulation of lipid in liver is significantly lower as compared to those in the higher dose level group exhibiting markedly elevated lipid levels. Although fatty acid biogenesis was inhibited after administration of 3.5 mg of tetracycline hydrochloride, synthesis of triglyceride was found to be stimulated in the liver of rats, which indicates that in this condition fatty acid portion of the triglyceride come almost exclusively from preformed fatty acid from the plasma.

In an attempt to obtain further information on the inhibitory effect of tetracycline on fatty acid synthesis in the liver of rats receiving 3.5 mg of antibiotic daily, the rates of incorporation of 2-C^{14}-acetate and 2-C^{14}-malonate into long chain fatty acid by liver slices were measured. It appeared from the results (Table 2) that even after 7 days of treatment of antibiotic, the rate of incorporation of radioacetate is considerably depressed. There was, however, no appreciable difference in the conversion of malonate to fatty acids, between the control and the tetracycline-treated animals. The inhibition of fatty acid biosynthesis in liver of tetracycline-treated animals must, therfore, take place at the pre-malonate stage, probably in the carboxylation of acetate to malonate. The effect of tetracycline in reducing the rate of fatty acid synthesis may be due to its action on the enzyme, acetyl COA car-

boxylase, which happens to be a key enzyme to the regulation of fatty acid synthesis in mammalian system. How tetracycline inhibits the carboxylation of acetate to malonate remains to be established.

Summary

Fatty acid biogenesis in rat liver is stimulated after administration of tetracycline hydrochoride at a low level of therapeutic recommendation, while, it is markedly inhibited when the dose was increased to high therapeutic level. However, there was no appreciable difference in conversion of 2-C^{14}-malonate to long chain fatty acids in liver slices of control and tetracycline-treated rats, which indicates that the inhibition of the fatty acids biosynthesis must, therefore, take place at the pre-malonate stage. The biosynthesis of cholesterol is lowered by tetracycline, irrespective of the doses of the drug administered.

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