STUDIES ON THE EFFECT OF ADMINISTRATION OF TETRACYCLINE ON FREE FATTY ACID METABOLISM IN ADRENALECTOMISED AND CONTROL RATS

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Administration of therapeutic doses of tetracycline hydrochloride to rats results in increased accumulation of triglyceride in the liver. Phospholipid concentration of the liver is decreased under such conditions, whereas both free and esterified cholesterol levels remain unaffected. Elevation of the free fatty acid level of plasma, induced by tetracycline administration, is due to increased mobilisation of free fatty acids from adipose tissue. The antibiotic was found to be ineffective in stimulating the mobilisation of free fatty acids from the epididymal fatpad of adrenalectomised rats.

The origin of fatty acids deposited as liver triglyceride remains speculative to a certain extent. HORNING *et al.*[1] demonstrated that after carbon tetrachloride treatment to rats, excessive accumulation of liver triglyceride results, due mostly to an increased mobilisation of free fatty acids (FFA) from adipose tissue. Liver triglyceride and plasma FFA levels are also elevated by stimulation of the pituitary-adrenal axis, and these changes are considerably depressed in adrenalectomised animals. LEPPER *et al.*[2] observed severe fatty infiltration of liver in experimental animals after intraperitoneal administration of tetracycline. Chlortetracycline treatment, on the other hand, results in increased accumulation of glycerides in the liver of mice.[3] Since the hepatic uptake of FFA is a direct function of plasma-concentration[4] and since increased mobilisation of unesterified fatty acids from adipose tissue occurrs after the administration of certain drugs[5,6], the present study describes the effect of administration of therapeutic doses of tetracycline on different lipid categories of liver, plasma FFA concentration as well as the release of FFA from adipose tissue of adrenalectomised and control rats in order to study the effect of this drug on the increased lipid accumulation in the liver.

Materials and Methods

Tetracycline hydrochloride was obtained from Standard Pharmaceuticals Ltd., Calcutta. Bovine Plasma albumin was purchased from Armour Pharmaceutical Co., England and Digitonin and Chromotropic acid-Na salt from E. Merck, W. Germany. Agla Micrometer Syringe was obtained from Burroughs Wellcome Laboratories, England.

Adult male albino rats weighing 100~120 g were kept on stock ration and the animals were allowed to feed *ad libitum*. Tetracycline hydrochloride, in aqueous solution, was

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administered intramuscularly daily, and the maximum and minimum dose-levels of therapeutic recommendation of the drug were used during the course of investigation. Blood was drawn directly from heart; the liver and the epidymal fat pad were saved and stored in ice-cold condition. Total lipid of the liver of experimental rats was extracted by the method followed by GERSHBEIN[7]. The estimation of free and esterified cholesterol was carried out by a modified procedure[8] of SCHOENHEIMER and SPERRY. Direct estimation of liver triglyceride was carried out according to VAN HANDEL and ZILVERSMIT[9] using the chromotropic acid reagent, while phospholipid was determined according to CHEN *et al.*[10]

In vitro incubation of epididymal fat pad of control and drug-treated rats was carried out in KREBES-bicarbonate buffer in the presence of Bovine plasma albumin according to GORDON and CHERKES.[11] The release of FFA from adipose tissue and plasma FFA was measured following the procedure of DOLE[12]. Adrenalectomy was performed under ether anaesthesia, and the animals were kept on glucose saline for a period of 2 days after which they were allowed to take stock ration and normal saline for a period of 3 days. The rats were sacrificed three hours after a single dose administration of 3.5 mg of tetracycline hydrochloride per 100 g of body weight of the animal.

Results and Discussion

Tetracycline treatment for a prolonged period of 4 weeks results in significant elevation of liver triglyceride levels at either dose levels of therapeutic recommendation (Table 1), the effect being more pronounced at the 3.5 mg per 100 g level of drug administration to rats. Almost five fold increase in neutral lipid content in liver following a daily administration of 3.5 mg dose level of tetracycline hydrochloride for a period of 4 weeks seems to be mainly responsible for severe fatty infiltration, observed after tetracycline treatment of the animals. The results clearly indicate that the degree of triglyceride deposition in the liver of rats, depends upon the dose of the antibiotic administered. When the drug was applied at low therapeutic level, the increment of neutral lipid deposition was about thrice as much as

Table 1. Lipid distribution of liver tissue of control and tetracycline treated rats.

	Number of rats	Duration of treatment	Dose of daily tetracycline (mg/100 g)	Liver lipids (mg/g of tissue)			
				Cholesterol		Triglyceride	Phospholipids
				Ester	Free		
Control	(12)			2.40±0.6	1.96±0.2	8.9±0.36	14 ±0.8
Tetracycline	(7)	4 weeks	1.5	2.64±0.3	2.0 ±0.12	23.9±0.15	12.4±0.98
Tetracycline	(6)	4 weeks	3.5	2.52±0.5	1.5 ±0.11	41.1±2.6	11.7±0.70
Tetracycline	(6)	7 days	3.5	2.6 ±0.2	1.8 ±0.22	14.2±0.4	12.1±0.14

Table 2. Effect of tetracycline on plasma free fatty acid level and rate of release of free fatty acid from adipose tissue.

	Number of rats	Duration of treatment	Daily dose of tetracycline (mg/100 g)	Plasma FFA (μE/ml)	Net release of FFA from adipose tissue (μm/g/hr)
Control	(9)	—	—	0.463±0.042	2.29 ±0.17
Tetracycline	(7)	4 weeks	1.5	0.572±0.053	3.247±0.14
Tetracycline	(6)	4 weeks	3.5	1.315±0.16	5.2 ±0.47
Tetracycline	(6)	7 days	3.5	0.87 ±0.11	4.73 ±0.5

Table 3. Effect of tetracycline administration to control and adrenalectomised rats on plasma FFA and net release of FFA from adipose tissue. (Drug treatment was made 3 hours before sacrifice.)

Group of animals	Number of rats	Plasma FFA (μE/ml)	Net release from adipose tissue (μm/g/hr)
Control	(6)	0.44 ±0.08	2.19±0.2
Tetracycline (3.5 mg/100 g)	(5)	0.82 ±0.13	3.92±0.45
Adrenalectomised	(6)	0.264±0.02	1.52±0.02
Adrenalectomised tetracycline	(7)	0.260±0.02	1.60±0.03

the control value. Increased accumulation of triglyceride in the liver of rats can also be precipitated as early as seven days after the beginning of drug treatment. The phospholipid contents of the livers of drug-treated rats are decreased to a significant extent, irrespective of the doses of the drug used. At 1.5 mg level of drug administration the depression of phospholipid was about 11 % while the antibiotic, when administered for a period of 4 weeks at higher level of therapeutic recommendation, supressed the phospholipid contents by about 16.0 % relative to the control. The esterified and free cholesterol contents of the liver of the drug treated animals remained almost unchanged during the course of investigation.

The administration of tetracycline hydrochloride to rats results in a significant increase in FFA-level in plasma (Table 2). Almost a three-fold rise in FFA content in plasma is noticed when the drug was introduced for a period of 4 weeks at 3.5 mg level, while at 1.5 mg level of drug administration, the increment is nearly 60 % over the control.

The change in concentration of FFA level of plasma is the resultant of two factors, mainly-an increased influx of FFA into the blood stream from fat-depot and a decreased rate of utilization of FFA in the body. It might be expected that the defect in fatty acid oxidation in tetracycline-treated rats, may lead to an increased FFA level in plasma. Though the effect of tetracycline on the *in vivo* oxidation of fatty acids in experimental rats has not yet been demonstrated, studies on the oxidation of fatty acid in mitochondrial preparation[13] in the presence of tetracycline revealed that the drug may considerably depress the catabolism. Increased mobilization of FFA from adipose tissue may give rise to high FFA level in plasma. In tetracycline-treated rats, the release of FFA from adipose tissue has been studied *in vitro*. The tissue FFA concentration has been simultaneously measured, before and after, in *in vitro* incubation of adipose tissue in order to determine the net release of FFA to the plasma. It is evident from the result that tetracycline stimulates lipolytic breakdown of adipose tissue triglyceride which may account for the increase in plasma FFA-level, although the antibiotic effect on catabolism of FFA studied in mitochondrial preparation cannot be completely ruled out as a factor contributing to observed rise in plasma FFA concentration.

The effect of a single dose administration of tetracycline (3.5 mg/100 g body weight) to control and adrenalectomised rats on the rate of release of FFA from adipose tissue is shown in Table 3. The results indicate that even after a single dose administration of tetracycline to rats, plasma FFA concentration is raised

considerably, while the drug is ineffective to induce such changes in adrenaletomised animals. Neither is there any significant difference in the rate of release of fatty acids from adipose tissue, the rate of mobilization of depot fat being considerably lower in tetracycline-treated animals, which have been subjected to adrenalectomy. It should be noted that tetracycline stimulates[14] incorporation of FFA into triglycerides in the liver of rats, and the stimulating effect of the drug is abolished when the drug was administered to adrenalectomised animals. Therefore, tetracycline besides stimulating the triglyceride synthesis in the liver, enhances the release of FFA from adipose tissue, elevating plasma FFA concentration, whereas extirpation of the adrenal glands results in impaired release of FFA from depot fat and thus limits its apply for transformation into triglycerides inspite of administration of this antibiotic to the animal.

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