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The effect of vitamin B_{12} on tetracycline-induced fatty liver

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With 3 tables

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Fatty change in the liver resulting from parenteral administration of tetracycline has been well documented from clinical experiences in man (24) and from experimental observations in laboratory animals (17, 26).

Electron-microscopic observations of hepatocytes gave indication that steatosis was induced by i.p. administration of high levels of tetracycline.

In rats treated with 100 mg/kg, typical fatty change was produced when the treatment was increased to 300 mg/kg; fine fat droplets also accumulated in the space of Disse (10).

Within 6 hrs of administration of steatogen (Ccl_4) there was a marked fall in liver vitamin B_{12} levels (15). *Kasbekar* et al. (15) suggested that the steatotic poison probably acts by provoking a temporary release of vitamin B_{12} from cellular particles to plasma.

Protection by vitamin B_{12} against fat accumulation in the liver was observed in experimental liver injury by carbon tetrachloride (21) and in thyrotoxic animals (14).

It was found that thyrotoxicosis results also in a vitamin B_{12} deficiency in blood and in liver and its mitochondrial fraction (14).

The fundamental metabolic deviation causing fatty degeneration has not yet been well understood. An attempt has been made here to show the major derangements preceding fat accumulation by tetracycline.

Materials and methods

Male and female Sprague-Dawley rats weighing 85–100 g were divided into four different groups.

First group was the control group.

Groups 2 and 3 were treated with three intraperitoneal injections of tetracycline (120 mg or 250 mg/kg).

Group 4 was treated with tetracycline plus vitamin B_{12}. Vitamin B_{12} was administered (i.m.) 3 hours before tetracycline (i.p.) three times in three consecutive days.

The animals were killed in the next day after the 3rd injection, samples of fasting blood were taken for lipid analysis.

The livers were carefully dissected out, and weighed pieces were homogenized in a glass homogenizer. The total lipid was extracted with chloroform-methanol (2:1) according to *Folch* (9).

Aliquots of the extract were used for estimation of cholesterol, triglycerides and phospholipids.

Total cholesterol was estimated in the liver by the method of Sackett (22) and in the serum by cholesterol-kit reagents of Merk.

Triglycerides in both serum and liver were estimated by triglycerides-kit reagents of BioMérièux.

Phospholipids was estimated in both serum and liver according to the method of *King* (16).

The turbidemetric method of serum total lipids was used by total-lipid kit reagents of BioMérièux.

Results and discussion

Treatment of Sprague Dawley rats with 120 or 250 mg tetracycline per kg body wt. per day for two or three days caused fatty liver in 75 % of the animals studied. The accumulated lipid was mainly triglycerides (Tables 1, 2).

These doses are approximately twice and four times the recommended maximum dose for man (18).

In rats treated with 120 mg/kg tetracycline, the liver total cholesterol increased and the phospholipids tend to decrease, while serum total cholesterol and phospholipids of treated animals were nearly the same as those of untreated control (tables 2, 3).

Studies with H-labeled palmitic acid indicated that tetracycline may activate phospholipases so that hepatic triglycerides are synthesized, while phospholipids are degraded (13).

In the present work no direct relationship was observed between dose of tetracycline and hepatic accumulation of triglycerides (table 1). Although livers of rats treated with the high dose of tetracycline appeared uniformly pale yellow.

Elevated serum triglyceride was found predominantly in rats treated with the small dose of tetracycline, while there was no obvious difference between serum triglyceride of rats treated with the high dose of tetracycline and control rats.

Table 1.

	Control	Tetracycline	
		120 mg/kg	250 mg/kg
Liver total Lipid mg/%	3.86 ± 0.47 (10)	8.65*) ± 1.60 (8)	7.8*) ± 1.6 (6)
Liver T.G. mg/g	5.22 ± 1.17 (10)	44.49*) ± 6.83 (6)	40.5*) ± 15.6 (6)
Serum T.G. mg/%	25.45 ± 5.20 (9)	90.7*) ± 23.3 (8)	24.4 ± 8.4 (5)

*) Significant

Table 2. Liver lipid levels.

	Total lipid (mg/g liver)	Triglycerides (mg/g liver)	Cholesterol (mg/100 g liver	Phospho-lipids mg/g
Control	3.86 ± 0.47 (10)	5.22 ± 1.17 (10)	186.26 ± 43.65 (7)	21.12 ± 5.02 (7)
Tetracycline 120 mg/kg	8.65 ± 1.64 (8)	44.49 ± 6.83 (6)	219.51 ± 93.40 (10)	16.99 4.24 (10)
P	< .05	< .05	> .05	> .05
Tetracycline + Vit. B_{12}	5.80 ± 1.22 (10)	19.12 ± 7.92 (10)	300.26 ± 120.80 (14)	18.24 ± 3.13 (13)
P	< 0.5	< 0.5	< 0.5	< 0.5

Thus the small dose of tetracycline produced fatty liver without affecting the rate of secretion of hepatic triglycerides, while the high dose caused a block in the release of hepatic triglycerides into plasma.

Fatty liver has been viewed by *Lombardi* (19) as the result of an imbalance between the rate of synthesis and the rate of utilization of hepatic triglyceride. Under this circumstance the accumulation of triglyceride in the hepatocytes is accompanied by lowered concentrations of triglycerides and lipoproteins in the plasma. The probability that a block in the release of lipoprotein into plasma would be a major mechanism in fatty liver was the reason for several studies of the effect of tetracycline on protein synthesis.

Yeh and *Shils* (25) found that tetracycline decreased incorporation of labeled amino acids into proteins in a number of organs.

Table 3. Serum lipid levels (mg %).

	Serum total lipid (mg/dl)	Serum triglycer-ides (mg/dl)	Serum total cholesterol (mg/dl)	Serum phospholipids (mg/dl)
Control	24.77 ± 52.8 (7)	25.45 ± 5.20 (9)	59.68 8.80 (8)	141.33 ± 38.20 (8)
Tetracycline 120 mg/kg	388.8 ± 48.0 (9)	90.7 ± 23.3 (8)	57.21 ± 20.50 (9)	149.8 ± 34.2 (12)
P	< .05	< .05	> .05	> .05
Tetracycline + Vit. B_{12}	329.55 ± 60.40 (12)	49.22 ± 16.10 (12)	57.36 ± 11.50 (13)	142.34 ± 39.60 (8)
	< 0.5	< 0.5	> 0.5	> 0.5

Evidence of impaired secretion of triglycerides and very low density lipoprotein (VLDL) was further substantiated by *Breen* et al. (3) in a study of perfused livers from rats treated with tetracycline. The specific nature of the block in triglyceride or VLDL synthesis and secretion remains uncertain.

Recently, *Gray* et al. (10) using the electron microscope studied the effect of tetracycline on ultrastructure and lipoprotein secretion in rat hepatocyte. They found that fat droplets of 0.1–0.5 μm accumulated in the space of Disse of hepatocytes in rats treated i.p. with 300 mg/kg of tetracycline hydrochloride.

This change was not observed in similar rats treated with 100 mg/kg.

One hour after an injection of 300 mg/kg, the antibiotic concentration was 10 times greater in the liver than in the serum. In rats treated with 100 mg/kg, no concentrations above 200 μg g were observed during a 48 hr postinjection period.

In the hepatocyte of rats treated with 300 mg/kg it was presumed that as the antibiotic concentration rose above 200 μg/g nascent very low density lipoprotein complexed rapidly with it. The complexed lipoprotein was channeled in dilated smooth endoplasmic reticulum (SER) in the manner of liposomes to the sinusoidal surface and discharged from vesicles into the space of Disse.

Thus in the present work the high blood and tissue concentrations of tetracycline play a prime role in the block of hepatic secretion of triglycerides in rats treated with 250 mg per kg.

Nevertheless *Breen* et al. (4) found that depressed hepatic secretion of triglyceride accounted only for 30–50 % of accumulated hepatic triglyceride, indicating that additional mechanisms must be involved in the production of the triglyceride-rich fatty liver in response to tetracycline.

Where protection by vitamin B_{12} was studied in the present work, the vitamin was given i.m. (50 μg/animal) 3 hours before the injection of 120 mg tetracycline per kg body wt.

Lipid abnormalities caused by tetracycline improved by Vitamin B_{12}. Thus both hepatic and serum total lipid and triglycerides were significantly lower than those of rats treated with tetracycline, although hepatic total cholesterol was significantly increased as in case of tetracycline only. Hepatic and serum phospholipid were nearly the same as those of the control (tables 2, 3).

Reports have appeared in the literature reviewed by *Arnstein* (1, 2) suggesting that vitamin B_{12} might play some part in the metabolism of lipids by animals.

Ferguson, Rigdon, and *Couch* (8) had reported histological evidence for the occurrence of fatty livers in the vitamin B_{12}-deficient chick embryo.

In rabbits with acute hepatic injury due to CCl_4, B_{12} showed a marked effect in preventing increase in liver fat content from the injury. In normal rabbits B_{12} had not any remarkable influence on the liver fat content (20).

In young rats on a steatogenic diet from weaning vitamin B_{12} exerts a lipotropic action to the 60th day of the experiment. In adult rats the lipotropic effect is not seen with vitamin B_{12} alone, but only if L-cystine is added to the vitamin (6).

The lipotropic effects of vitamin B_{12} varied with the amount administered, the route of administration, and the diet taken (11).

DuBuy and *Showacre* (7) demonstrated by use of the fluorescent property of tetracycline that these antobiotics combine specifically with the mitochondria of living cells, either in tissue culture or in fresh preparations from various organs. They also confirmed that oxidative phosphorylation was decreased by tetracycline under the conditions of their studies, but that oxygen uptake of mitochondria was not changed. Subsequently, degenerative changes were verified in the mitochondria of Hela cells exposed to an antibiotic concentration of 100 μg/ml and higher (12).

Impairment of mitochondrial integrity could either arise from or be the cause of depletion in liver vitamin B_{12} and glutathione. A role for the sulphydryl group and glutathione in the protection of cellular morphology has been postulated (23).

Butturini et al. (5) suggested that vitamin B_{12} may exert its anti-steatogenic action by increase in glutathione.

According to *Kasbekar* (15) protection against fat accumulation by vitamin B_{12} is non-specific and may be due to an effect on the maintenance of mitochondrial integrity.

Summary

The effect of vitamin B_{12} on the metabolic alterations due to tetracycline toxicity was studied experimentally on laboratory animals.

Treatment of Sprague-Dawley rats with 120 or 250 mg tetracycline (i.p.) per kg per day for two or three days caused an accumulation of lipids, mainly triglycerides in the liver of 75 % of animals studied, while phospholipid level tend to decrease.

These doses are approximately twice and four times the recommended maximum dose for man.

In the present work no direct relationship was observed between dose of tetracycline and hepatic accumulation of triglyceride, although livers of rats treated with 250 mg tetracycline/kg appeared uniformly pale yellow.

Elevated serum triglyceride was found predominantly in rats treated with 120 mg/kg, while there was no obvious difference between serum triglyceride of rats treated with 250 mg tetracycline and control rats, indicating a block in the release of hepatic triglycerides.

Where protection by vitamin B_{12} was studied, the vitamin was given i.m. (50 μg/animal) 3 hours before the injection of 120 mg tetracycline per kg.

There was a good evidence that lipid abnormalities caused by tetracycline improved by vitamin B_{12}.

Thus both hepatic and serum total lipid and triglycerides were significantly lower than those of rats treated with tetracycline, although hepatic total cholesterol was significantly increased as in case of tetracycline only.

References

1. *Arnstein, H. R. V.:* Symp. biochem. Soc. **13,** 92 (1955). – 2. *Arnstein, H. R. V.:* Proc. 4th Int. Congr. Biochem., Vienna, **11,** 286 (1958). – 3. *Breen, K., S. Schenker, M. Heimberg:* Biochim. Biophys. Acta **270,** 74 (1972). – 4. *Breen, K. J., S. Schenker, M. Heimberg:* Gastroenterology **69, 714** (1975). – 5. *Batturini, U., A. Baronchelli, F. Bagnaschi:* Boll. Soc. ital. biol. Sper. **28,** 1420 (1952). – 6. *Dessi, P., L. L. Barbieri, M. A. Brunelli, A. M. Gianni:* Arch. Sci. biol. **38,** 286 (1954). – 7. *Dubug, H. G., J. L. Showacre:* Science **133,** 196 (1961). – 8. *Ferguson, Rigdon, Couch:* (1955) Quoted from *Moore, H. J., B. M. Doran.* Biochem. J. **84,** 506 (1962). – 9. *Folch, J. M., G. H. Lees, S. Sloane:* J. Biol. Chem. **226,** 497 (1957). – 10. *Gray, J. E., R. N. Weaver, P.*

Skinner, J. Mathews, C. E. Day, K. Stern: Toxicology and Applied Pharmacology **30,** 317 (1974). – 11. *Josef, S., M. Frantisek:* Progr. Biochem Pharmacol. **2,** 459 (1967). – 12. *Journey, L. J., M. N. Goldstein:* Cancer Res. **23,** 551 (1963). – 13. *Kamen, K.:* Zhivol-noud, Nauki **9,** 11 (1972). – 14. *Kasbekar, D. K., W. V. Lavate, D. V. Rege, A. Sreenivasan:* Biochem. J. **72,** 374 (1959a). – 15. *Kasbekar, D. K., W. V. Lavate, D. V. Rege, A. Sreenivasan:* Biochem. J. **72,** 384 (1959b). – 16. *King, E. J., I. Wooton:* Microanalysis in Medical Biochemistry 3 rd. ed. (London) (1956). – 17. *Lewis, M., S. Schenker, B. Combes:* Amer. J. Dig. Dis. **12,** 429 (1967). – 18. *Likins, R. C., G. A. Pakis:* Nature **207,** 1394 (1965). – 19. *Lombardi, B.:* Lab. Invest. **15,** 1 (1966). – 20. *Matsushita, Y.:* Naika Hôkan **3,** 657 (1956). – 21. *Rege, D. V.,* Ph.D. Thesis, University of Bombay (1953). – 22. *Sackett, G. E.:* J. Biol. Chem. **64,** 203 (1925). – 23. *Tapley, D. F., C. Cooper:* J. Biol. Chem. **222,** 341 (1956). – 24. *Wruble, L. D., A. J. Cummins:* Amer. J. Dig. Dis. **10,** 742 (1965). – 25. *Yeh, S. D. J., M. E. Shils:* Proc. Soc. Exp. Biol. Med. **121,** 729 (1966). – 26. *Zussman, W. V.:* Anat. Rec. **162,** 301 (1968).

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