PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 868-872 (1974)

Biochemical Indices of Tetracycline Hepatic Injury in Rats (38458)

BRUCE A. SHAUER, LORINC LUKACS, AND HYMAN J. ZIMMERMAN

Medical Service, Veterans Administration Hospital and the Department of Medicine, George Washington University School of Medicine, Washington, DC

A serious form of clinical hepatotoxicity can result from the intravenous administration of chloretetracycline, oxytetracycline, and tetracycline (1–8). Evidences of hepatic injury have included hyperbilirubinemia and, in the few instances reported, only modest elevation of the serum, glutamic oxalactic transaminase (SGOT) and alkaline phosphatase values (5–8). The hepatic lesion consists of small droplet-type of fatty metamorphosis with persistence of lobular architecture, and but little necrosis or inflammatory response (9). Administration of chloretetracycline (10), oxytetracycline (10), and tetracycline (11) to experimental animals can produce a similar small-droplet fatty metamorphosis (10–14). Data on the biochemical reflections of the hepatic injury in humans and experimental animals, however, are relatively sparse. Accordingly, the present study was designed to demonstrate the effects of graded, daily doses of tetracycline on biochemical measures of hepatic injury, with particular emphasis on the serum levels of glutamic oxalacetic [SGOT, L-aspartate: 2-oxoglutarate aminotransferase (2.6.1.1)]; and glutamic pyruvic [SGPT, L-alanine: 2-oxoglutarate aminotransferase (2.6.1.1)] transaminases.

Materials and Methods. Animals. Two hundred female Wistar rats with a weight range of 160–200 g were used. The animals were maintained on water and Purina Chow *ad libitum*.

Drugs and related preparations. Tetracycline hydrochloride (Achromycin, Lederle Pearl River, NY) supplied in vials containing 500 mg tetracycline and 1250 mg of ascorbic acid was dissolved daily in isotonic saline to provide a concentration of 100 mg tetracycline/ml. Other solutions used included ascorbic acid for injection into controls (USP Cevalin 500 mg/ml, Eli Lilly, Indianapolis, IN); bacteriostatic sodium chloride (USP, Ambot Solution; Cutter Laboratories Inc., Berkely, CA); nembutal sodium (sodium pentobarbital) 50 mg/ml, Abbott Laboratories, Chicago, IL.

Procedure. The experimental protocol is shown in Table I. The animals were divided into 10 groups; four groups received graded doses of tetracycline (TCL); four groups received corresponding doses of ascorbic acid (AA) as controls; one group received saline injections; and one control group remained uninjected. Animals of each group received the doses indicated and were sacrificed at the time shown in Table I. All injections were intraperitoneal.

Blood samples for biochemical studies were obtained by cardiac puncture without anticoagulant. Animals were then sacrificed by exsanguination and a sample of liver was taken for routine hematoxylin and eosin and oil red O stains as well for electron microscopy.

Methods of analysis. The serum samples from the animals of each group were pooled, and the measurements performed in triplicate. The values given are the means of these triplicate determinations. Serum separated after drawing of the blood was assayed for the SGOT, SGPT, GD [glutamic dehydrogenase, L-glutamate: NAD^+ oxidoreductase (1.4.1.2)], LD [lactic dehydrogenase, L-lactate: NAD^+ oxidoreductase (1.1.27)], acid phosphatase (3.1.3.2), alkaline phosphatase (3.1.3.1), β-glucuronidase (3.2.1.31), bilirubin, and tetracycline levels. The SGOT determination was done by the method of Karmen (15) as modified by Steinberg *et al.* (16). The SGPT was done according to the method of Wroblewski and LaDue (17). The glutamic dehydrogenase (a measure of mitochondrial injury) was measured by the modification of the method of Beiswenherz (18). The acid and alkaline phosphatase levels were measured by the methods of Bodansky (19). The β-glucuronidase was measured by the method of

TABLE I. Protocol for Experiments.[a]

GRP	Treatment: TCL[c] (mg/kg)	Treatment: AA[d] (mg/kg)	Treatment: Saline (ml)	Sacrifice at hr after first injection[b]: 24	48	72	96
1	50	125	0.09	5	5	5	5
2	0	125	0	4[e]	5	5	5
3	100	250	0.18	5	5	5	4[e]
4	0	250	0	4[e]	5	5	5
5	200	500	0.36	5	5	6[f]	0
6	0	500	0	5	4[e]	4[e]	0
7	300	750	0.54	5	6[f]	0	0
8	0	750	0	5	4[e]	0	0
9	0	750	0.54	5	5	5	4
10	0	0	0	5	5	5	5

[a] Groups 1, 3, 5, and 7—TCL test groups. Groups 2, 4, 6, and 8—AA control, received amount of AA contained in TCL preparation.

[b] All animals that were not sacrificed received their respective injection daily—for example 96 hr group received injection at time 0, 24, 48, 72 hr and was sacrificed at 96 hr.

Group 5—only six of 10 remaining animals survived at 72 hr; accordingly, this group and its controls (group 6) were sacrificed at that time.

Group 7—only six of 15 animals survived at 48 hr; accordingly, this group and its controls (group 8) were sacrificed at this time.

[c] TCL = tetracycline.

[d] AA = ascorbic acid.

[e] Instead of 5 because animal found dead in cage or died after nembutal anesthesia (0.10 ml).

[f] 6 instead of 5 because surviving group was pooled.

Fishman (20), and the serum bilirubin was measured by the method of Malloy and Evelyn (21). The tetracycline was assayed biologically by the method outlined by Grove and Randall (22).

Results. Values for serum enzymes in the several groups are listed in Table II. The most striking abnormalities were observed with the levels of the SGOT which showed a direct relationship with the dose of TCL administered. Values for the SGPT were increased above the respective controls only at doses of 200 mg/kg or more. Values for GD, acid phosphatase, alkaline phosphatase, β-glucuronidase were no greater in the animals that received TCL than in the respective controls. Values for bilirubin remained below 1 mg/100 ml. As shown in Table II, TCL levels in the blood reflected the doses administered.

At all dose levels employed TCL led to SGOT values higher than those of the respective controls as early as 24 hr after administration. Daily administration led to peak values at 48 hr; beyond this period there was little further increase as shown in the 200-mg/kg group, or a decline (50- and 100-mg dose level) despite continued administration of the drug (Table II). There were no values beyond 48 hr for animals that had been receiving 300 mg/kg TCL, since the death rate beyond 48 hr was too great.

Values for SGPT remained essentially unchanged throughout the experiment in animals which received doses of 50 or 100 mg/kg. At the 200-mg/kg dose there was a threefold increase in the SGPT at 24 hr. Despite continued daily administration of the drug, subsequent values for the SGPT were even lower than those of the first day (Table II).

Administration of ascorbic acid led to values that were somewhat higher than those of the saline or uninjected controls. The highest values in this group were observed at 48 hr.

Histologic changes observed in the several groups consisted of vacuolization typical for the tetracycline effect. These changes were seen in all the members of the group that received 200 and 300 mg/kg. The changes were modest at 24 hr and reached a maximum at 48 hr. In the animals that received 100 mg/kg there were no recognizable changes by light microscopy at 24 or 48 hr; however, a moderate degree of vacuoli-

TABLE II. Value for Serum Enzymes[a] in Rats Receiving Tetracycline (TCL), Ascorbic Acid (AA) at Various Intervals.

Interval from beginning of experiment	Treatment	SGOT	SGPT	GDH	Acid Phos	Alk Phos	Gluc	TCL (μg/ml)
	50 mg/kg TCL	222	23	1	2.65	32	2.2	0.30
	100 mg/kg TCL	351	26	3	1.70	25	1.5	0.70
	200 mg/kg TCL	417	99	1	0.8	21	2.0	9.8
	300 mg/kg TCL	514	80	2	1.4	28	1.7	19.8
	50 mg/kg AA	147	24	1	2.3	33	1.7	0
	100 mg/kg AA	130	20	2	—	36	1.3	0
24 hr	200 mg/kg AA	165	37	2	3.2	35	1.6	0
	300 mg/kg AA	124	25	1	3.8	29	1.2	0
	NaCl	123	22	2	2.5	30	1.4	0
	No injection	142	34	3	2.1	37	1.8	0
	50 mg/kg TCL	354	29	1	2.1	13	2.0	1.27
	100 mg/kg TCL	604	42	2	1.5	13	1.6	2.4
	200 mg/kg TCL	840	72	3	1.7	7	1.8	9.6
	300 mg/kg TCL	905	75	3	2.1	5	2.5	15.5
48 hr	50 mg/kg AA	232	22	2	2.1	8	2.0	0
	100 mg/kg AA	262	26	2	2.7	12	2.1	0
	200 mg/kg AA	198	22	2	2.3	17	1.7	0
	300 mg/kg AA	288	35	1	1.9	12	1.5	0
	NaCl	152	31	2	5.0	11	1.8	0
	No injection	124	41	2	3.0	10	1.7	0
	50 mg/kg TCL	467	36	1	1.9	9	2.0	2.00
	100 mg/kg TCL	515	49	1	3.1	12	1.6	3.10
	200 mg/kg	980	65	2	4.0	7	1.8	16.90
	50 mg/kg AA	193	26	1	1.8	13	1.9	0
72 hr	100 mg/kg	273	26	2	2.6	20	1.9	0
	200 mg/kg AA	245	29	3	3.1	21	1.6	0
	NaCl	185	40	3	2.8	15	2.0	0
	No injection	190	35	4	0.7	15	1.8	0
	50 mg/kg TCL	446	63	3	3.0	20	—	1.78
	100 mg/kg TCL	558	52	2	1.5	11	—	5.40
96 hr	50 mg/kg AA	252	26	2	1.9	19	—	0
	100 mg/kg AA	227	36	2	2.7	21	—	0
	NaCl	208	25	2	9	20	—	0
	No injection	187	34	4	6.5	20	—	0

[a] Abbreviations: SGOT, glutamic oxalacetic transaminase; SGPT, glutamic pyruvic transaminase; GDH, glutamic dehydrogenase; Acid Phos, acid phosphatase; Alk Phos, alkaline phosphatase; Gluc, β-glucuronidase; SGOT and SGPT in Karmen (15) units. GDH and glucuronidase in IU/ml. Alkaline and acid phosphatase in Bodansky (19) units.

zation was seen at 72 and at 96 hr. The animals that received 50 mg/kg showed no changes identifiable by light microscopy, at any time during the study.

Electron microscopic studies are to be reported separately; however, ultrastructural evidence of fat accumulation was observed at all dose levels, roughly proportionate to the dose. No mitochondrial abnormalities were observed in these preliminary studies.

Discussion. The data of the present study confirm the available information on serum enzyme changes as a reflection of the hepatic injury of TCL. Values for SGOT were the most sensitive reflection of injury and showed a relationship to dose of drug. Elevations were slight at 50 mg/kg but appreciable at 100 mg or more. These values were maximal by 48 hr, by which time two doses of the drug had been injected. The difference in levels between successive doses were significant as shown by regression curve analysis (23) (Fig. 1), with the dose effect most clearly apparent at

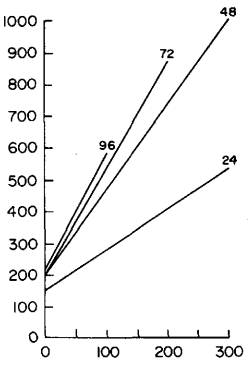

FIG. 1. Linear regressions of SGOT on dose of TCL for each of four time periods. Statistical analysis of relationship of level of SGOT to dose of TCL at 24, 48, 72, and 96 hr after initial dose. Correlation coefficient, r; slope, b (increase in SGOT per unit of TCL); standard deviation of the slope, S_b.

	TCL24	TCL48	TCL72	TCL96
N	6	6	5	4
r	0.97	0.96	0.99	0.98
b	1.28	2.70	3.83	3.73
S_b	0.15	0.40	0.36	0.51

Comparison of slopes: 24 vs 48, $P < 0.005$; 24 vs 72, $P < 0.001$; 48 vs 72, $P = 0.06$ NS.

72 hr. Indeed, the SGOT values appear to be a more significant reflection of hepatic injury than were the histologic changes by light microscopy. At 50 mg/kg TCL there were no discernable histologic changes, although values for SGOT were higher than those of the respective control. Values for SGPT, however, were an insensitive measure of hepatic injury and only at the large dose of 200 mg/kg or more were there elevations of the level. Furthermore, while the SGOT values rose progressively over the 48-hr period reaching a peak at that time, the values for SGPT were only transiently elevated despite continued administration of the drug.

The serum enzyme response to tetracycline-induced hepatic injury differs clearly from that of hepatotoxin-induced necrosis and from that of nutritional fatty metamorphosis. Carbon tetrachloride, for example, leads to hepatic necrosis and striking elevations of the SGOT up to 100-fold the normal (24), and the values are increased within 3 hr of the first dose (24). The increases in levels of SGPT also are striking in carbon tetrachloride-induced necrosis (24). In contrast are the modest elevations of SGOT and even lower levels of SGPT induced by tetracycline. Fatty metamorphosis induced by a high-fat, low-choline diet even when marked yields little or no abnormality in SGOT and SGPT values (25).

The form of hepatic steatosis induced by tetracycline resembles that observed in Reye's Syndrome (26) (encephalopathy and fatty degeneration of the viscera), fatty liver of pregnancy (6), and the fatty liver induced by some hepatotoxins, *e.g.*, ethionine (27) or puromycin (28). All of these circumstances are associated with a microvesicular (fine-droplet) form of fatty metamorphosis. These entities also are characterized by modest elevations of the SGOT and SGPT not unlike those observed with tetracycline. It is also characteristic of the fatty liver of Reye's syndrome that bilirubin levels are usually only modestly elevated and deep jaundice is rare (26). Similarly, in the present study, bilirubin levels remained normal and in the reported instances of tetracycline-induced toxicity in patients (5–8) the degree of hyperbilirubinemia has usually been modest despite often great gravity of the total syndrome produced.

Summary. The intraperitoneal administration of tetracycline (TCL) to female Wistar rats in graded doses ranging from 100 to 300 mg/kg led to the development of small-droplet fatty metamorphosis by 24 hr and to modest, dose-related, increased serum levels of glutamic–oxalactic transaminase (SGOT). A dose of 50 mg/kg of TCL led to an increase in the SGOT level but not to demonstrable fatty metamorphosis. Serum levels of glutamic pyruvic transaminase were found to be less sensitive than those of SGOT as a reflection of the hepatic injury. Serum levels of glutamic dehydrogenase, acid and alkaline phosphatase, β-glucuronidase, and bilirubin remained normal.

1. Lepper, M. H., Zimmerman, H. J., Carroll, G., Caldwell, E. R., Jr., Spies, H. W., Wolfe, C. K., and Dowling, H. F., Arch. Intern. Med. **88,** 271 (1951).

2. Yesner, R., and Kunkel, P., Yale J. Biol. Med. **23,** 299 (1950).

3. Dowling, H. F., and Lepper, M. H., J. Amer. Med. Ass. **188,** 307 (1964).

4. Wrubler, L. D., Ladman, A. K., Britt, L. G., and Cummins, A. J., J. Amer. Med. Ass. **192,** 6 (1965).

5. Schultz, J. C., Adamson, J. S., Jr., Workman, W. W., and Norman, T. D., New Engl. J. Med. **269,** 999 (1963).

6. Kunelis, C. T., Peters, J. L., and Edmondson, H. A., Amer. J. Med. **38,** 359 (1965).

7. Whalley, P. J., Reuben, H. A., and Burton, C., J. Amer. Med. Ass. **189,** 103 (1964).

8. Peters, R. L., Edmondson, H. A., Mikkelsen, W. P.,

and Tatter, D., Amer. J. Surg. **113,** 622 (1967).

9. Davis, J., *in* "Drug Induced Disease" (L. Meyler and H. M. Peck, eds.). p. 103. Excerpta Medica Foundation, Amsterdam (1968).

10. Lepper, M. H., Wolfe, C. K., Zimmerman, H. J., Caldwell, E. R., Jr., Spies, H. W., and Dowling, H. F., Arch. Intern. Med. **88,** 284 (1951).

11. Seto, J. T., and Lepper, M. H., Antibiot. Chemother. **4,** 666 (1954).

12. Miller, S. E. P., MacSween, R. N., Glen, A. C., Tribedi, K., and Moore, F. M., Brit. J. Exp. Pathol. **48,** 51 (1967).

13. Steiner, G., Bradford, W., and Craig, J. M., Lab. Invest. **14,** 1456 (1965).

14. Shils, M. E., Clin. Pharmacol. Ther. **3,** 321 (1962).

15. Karmen, A., J. Clin. Invest. **34,** 131 (1955).

16. Steinberg, D., Baldwin, D., and Ostrow, B. H., J. Lab. Clin. Med. **48,** 144 (1956).

17. Wroblewski, F., and LaDue, J., Proc. Soc. Exp. Biol. Med. **91,** 569 (1956).

18. Beisenherz, G., Boltze, H. K., Bucher, T., Czok, R., Garbade, K. H., Meyer-Arendt, E., and Pfeider, G., Z. Naturforsch. **8B,** 555 (1953).

19. Bodansky, A., J. Biol. Chem. **101,** 93 (1933).

20. Fishman, W. H., J. Biol. Chem. **173,** 449 (1948).

21. Malloy, H. T., and Evelyn, K. A., J. Biol. Chem. **119,** 481 (1937).

22. Grove, D. C., and Randall, W. A., "Assay Methods of Antibiotics, A Laboratory Manual," p. 50. Medical Encyclopedia Inc., New York (1955).

23. Snedecor, G. W., and Cochran, W. C., "Statistical Methods," 6th ed. Iowa State University Press, Ames, IA (1967).

24. Zimmerman, H. J., Kodera, Y., and West, M., J. Lab. Clin. Med. **66,** 315 (1965).

25. Zimmerman, H. J., Kodera, Y., and West, M., J. Lab. Clin. Med. **66,** 324 (1965).

26. Reye, R. D. K., Morgan, G., and Baral, J., Lancet **2,** 749 (1965).

27. Farber, E., Simpson, M. V., and Tarver, H., J. Biol. Chem. **182,** 91 (1950).

28. Slater, T. F., and Sawyer, B. C., Biochem. Pharmacol. **15,** 1267 (1966).

29. Rees, K. R., and Sinha, K. P., J. Pathol. Bacteriol. **80,** 297 (1960).

30. Partin, J. C., Schubert, W. K., and Partin, J. S., New Engl. J. Med. **285,** 1339 (1943).

31. Moritz, M., and Snodgrass, P. J., Gastroenterology **62,** 93 (1972).

Received April 27, 1974. P.S.E.B.M., 1974, Vol. 147.