Zeitschrift für Ernährungswissenschaft

Journal of Nutritional Sciences · Journal des Sciences de la Nutrition

Band 16 Heft 4 Dezember 1977

Z. Ernährungswiss. **16**, 217–222 (1977)

ISSN 0044-264 X

National Research Centre, Dokki, Cairo,
and Faculty of Medicine, Cairo University (Egypt)

Biochemical changes under the effect of carbon tetrachloride intoxication

E. A. El-Dessoukey, R. Awadallah, and *S. El-Attar*

With 3 tables

(Received June 13, 1977)

Carbon tetrachloride is widely used as a solvent in the manufacture of rubber, paint, soaps and quartz industries (15). Absorbed carbon tetrachloride either by the lungs or by the intestinal tract (22, 19) was reported to affect different tissues including bone marrow, liver, brain, kidney, and pancreas (12).

It is generally agreed that the pathological changes of the liver such as central necrosis, fatty infiltration, and changes similar to acute yellow atrophy has been occurred in animals intoxicated by carbon tetrachloride (5, 13). Such pathological changes have proved to depend on the degree of exposure to the drug (8). Liver dysfunction would affect its ability to synthesize proteins at a normal rate, in particular serum albumin which is mainly produced by the hepatic parenchyma (7).

Carbon tetrachloride is well known to produce acute liver damage if given in single doses and cirrhosis if given repeatedly (9, 10). In such acute liver damage an increase in plasma iron and total iron binding capacity (TIBC) has been reported (17, 18). In the hepatic cells oxidative metabolism and the mechanism by which ferritin iron from serum iron incorporates into the liver are disturbed. Nevertheless, this process of iron transfer gain momentum upon liver regeneration and, eventually, exceeds the normal rate after a few days. Most of previous studies (11, 21) had reported changes concerned with iron metabolism in chronic and acute liver damage.

The aim of the present study is to evaluate the changes in the general features of protein and iron metabolism in rats with chronic and acute liver damage induced by carbon tetrachloride.

Material and methods

Albino rats of either sex and weighing between 120–150 g were used. They were kept in separate cages and were maintained on a balanced stock diet with water ad libitum. Animals were divided into three experimental groups.

The first group comprises 45 animals represented the chronic liver damage. This was subdivided into three subgroups namely I, II, and III of chronic liver damage according to the criteria of *Mangalik* et al. (10). These subgroups received intraperitoneal injections of 10 % carbon tetrachloride at 0.15 ml per 100 g body weight three times a week for 3, 6, and 9 weeks respectively.

The second group (15 rats) represented the model of acute liver cirrhosis according to that described by *Loh* et al. (9). The animals of this group were fasted for 16 hours before carbon tetrachloride injection which consisted of 0.3 ml of 10 % solution in liquid paraffin per 100 g body weight given intraperitoneally.

The third group (20 rats) served as a control group by receiving intraperitoneal injections of 0.3 ml per 100 g body weight of liquid paraffin alone.

Serum total protein and its simple electrophoretically separated fractions were determined by the method of *King* and *Wooton* (6). Blood haemoglobin was determined by *Wong* method (23) and haematocrit value by micro-haematocrit tubes. Reticulocytes, stained with new methylene blue, were counted under light microscope. Their numbers were expressed as percentage of the circulating red cells present (14). Plasma iron and total iron binding capacity were estimated by the method of *Rammsey* (16).

Results and discussion

Data describing the levels and percentage changes of serum total protein and its simple electrophoretically separated fractions in control and carbon tetrachloride intoxicated rats are represented in table 1 and 2.

Our data revealed a decline in the levels of serum total protein and albumin in groups of chronic and acute carbon tetrachloride intoxication. A state of hypoproteinemia and hypoalbuminemia may be observed in rats with severe conditions of carbon tetrachloride intoxication. Hypoproteinemia and hypoalbuminemia may be explained as a result of the unfavourable effect of carbon tetrachloride on the liver, and possibly other sites in the body are responsible for the synthesis of plasma proteins. Another contributing factor that may affect protein synthesis is the loss of appetite noticed in rats intoxicated with carbon tetrachloride. Food restriction has been reported to affect the rate of protein synthesis, particularly albumin (20).

An increase in the levels of $alpha_1$ and $alpha_2$ globulin in both chronic and acute carbon tetrachloride induced cirrhosis in rats were found. Such increase in $alpha_1$ and $alpha_2$ globulins encountered in our intoxicated rats agrees with data previously reported by *Gutman* (3) who emphasized the increase in serum alpha globulins with the decrease in albumin.

Furthermore, the finding of increased level of beta-globulin fraction demonstrated in the first, second and third grades as well as in acute carbon tetrachloride intoxication corresponds to the previous work of *Fukuoka* (2). Such increase may be attributed to the increased synthesis of beta globulin in the liver to compensate the lowering of serum albumin. Moreover, *Zinkel* et al. (24) reported an increase in the beta globulin during carbon-tetrachloride intoxication.

Table 1. Summary of data for serum total protein and its major fractions in control and carbon tetrachloride intoxication.

Item		T. pr.	Alb.	α_1-G.	α_2-G.	B-G.	γ-G.	T. Gl.	Alb./T. Gl.
Control	M	7.15	3.34	0.50	0.72	1.48	1.11	3.81	0.88
	S.E.	± 0.149	± 0.078	± 0.036	± 0.062	± 0.069	± 0.076	± 0.122	± 0.062
	P <								
Group I	M	6.89	2.90	0.56	0.77	1.59	1.07	3.99	0.73
	S.E.	± 0.167	± 0.067	± 0.032	± 0.064	± 0.061	± 0.076	± 0.184	± 0.054
	P <	0.15	0.025	0.15	0.15	0.15	0.15	0.15	0.15
Group II	M	6.74	2.67	0.64	0.79	1.62	1.02	4.07	0.65
	S.E.	± 0.178	± 0.097	± 0.058	± 0.054	± 0.068	± 0.068	± 0.196	± 0.066
	P <	0.025	0.005	0.025	0.10	0.15	0.15	0.15	0.025
Group III	M	6.48	2.25	0.75	0.86	1.68	0.94	4.23	0.53
	S.E.	± 0.184	± 0.086	± 0.041	± 0.048	± 0.073	± 0.089	± 0.178	± 0.078
	P <	0.025	0.005	0.005	0.025	0.025	0.10	0.025	0.005
Acute ccl_4	M	6.20	1.78	0.86	0.98	1.72	0.86	4.42	0.40
	S.E.	± 0.152	± 0.088	± 0.063	± 0.055	± 0.058	± 0.067	± 0.152	± 0.076
	P <	0.005	0.005	0.005	0.005	0.025	0.025	0.025	0.005

Table 2. Percentage average changes in serum total protein and its major fractions of carbon tetrachloride intoxicated rats relative to controls.

Item	T. pr.	Alb.	α_1-G.	α_2-G.
Group I	− 3.7	−13.2	+12.0	+ 6.9
Group II	− 5.8	−20.1	+28.0	+ 9.7
Group III	− 9.4	−22.7	+50.1	+19.4
Acute ccl_4	−13.3	−46.8	+72.0	+36.1

Item	B-G.	γ-G.	T. Gl.	Alb. / T. Gl.
Group I	+ 7.4	− 3.7	+ 4.7	−17.1
Group II	+ 9.4	− 8.2	+ 6.8	−26.2
Group III	+13.5	−15.4	+11.5	−39.8
Acute ccl_4	+16.2	−22.6	+16.0	−54.6

On the other hand, no significant change in the level of gamma globulin could be observed in grade I and II. A slight decrease in its level was found in the last groups. As the gamma globulin are synthesized in the reticuloendothelial system (20), the diminution found in our data could be attributed to the result of possible interference of carbon tetrachloride with the plasma lymphoid cells, responsible for antibody formation.

In addition, the observed decrease in albumin globulin ratio caused by the decrease of albumin as well as the increase in the total globulin levels.

As shown in table 3, there was a decrease in haematocrit values in rats with both chronic and acute liver damage induced by carbon tetrachloride, this might be attributed to an expansion in plasma volume. *Hall* (4) found that the decrease in haematocrit values associated with increase of plasma volume.

Furthermore, the haemoglobin of grade I cirrhosis was first raised to a level significantly higher than control value but dropped to about the control value level in grade II, and eventually in grade III fell below that of controls. In rats with acute liver damage, there was however no significant change in the haemoglobin concentration in the circulating blood. The changes of haemoglobin concentration in the three stages of chronic carbon tetrachloride liver damage and the calculated mean corpuscular haemoglobin concentrations (MCHC) of 38.2, 41.9 and 34.5 respectively, compared to control value of 35.2, suggest that compensatory mechanism such as increase in the red cell volume (1) might have taken place, particularly in grade I and II cirrhosis. However, these compensatory measures could no longer remain effective in grade III, since low mean corpuscular haemoglobin concentration was found.

No significant changes could be observed in reticulocytes count in all groups of chronic and acute carbon-tetrachloride intoxicated rats.

In acute carbon tretrachloride toxicity a highly significant elevation of plasma iron and total iron-binding capacity were observed (table 3). The high plasma-iron content could be suggested to the appearance of ferritin

Table 3. The changes in the haematological measurements at different phases of carbon tetrachloride intoxication.

Item		Control	Grade I	Grade II	Grade III	Acute ccl_4
Haematocrit	M	50.4	46.2	36.6	44.8	45.4
%	S.E.	± 1.826	± 1.472	± 2.126	± 1.997	± 1.859
	P <		0.10	0.005	0.025	0.025
Haemoglobin	M	13.33	14.88	13.18	10.74	13.48
(g/100 ml)	S.E.	± 0.539	± 0.646	± 0.568	± 0.531	± 0.411
	P <		0.15	0.15	0.025	0.15
MCHC	M	35.2	38.2	41.9	34.5	37.3
%	S.E.	± 1.442	± 1.684	± 1.944	± 1.364	± 1.764
	P <		0.15	0.10	0.15	0.15
Reticulocytes	M	1.62	1.95	1.64	2.01	1.92
%	S.E.	± 0.191	± 0.117	± 0.111	± 0.131	± 0.094
	P <		0.10	0.15	0.05	0.10
Plasma iron	M	158	146	178	164	432
(μg/100 ml)	S.E.	± 5.144	± 5.228	± 5.176	± 4.876	± 11.738
	P <		0.15	0.10	0.15	0.05
Plasma TI BC	M	286	298	326	306	873
(μg/100 ml)	S.E.	± 8.542	± 7.654	± 9.468	± 8.782	± 24.126
	P <		0.15	0.15	0.025	0.005

MCHC: denotes mean corpuscular haemoglobin concentrations.

in the systemic circulation resulting from the disintegration of hepatic cells. On the other hand, plasma-iron and total iron-binding capacity were not elevated in rats with various degrees of chronic liver damage.

Summary

The present study deals with investigations on the general features of protein and iron metabolism under several conditions of chronic and acute liver damage induced by carbon tetrachloride. Data revealed a drop in the levels of serum total protein and albumin. The levels of alpha$_1$, alpha$_2$ and beta globulins were found to be increased. Gamma fraction was found to be proportionate with dosage accumulation and to varying extent.

There were changes in haematocrit and haemoglobin values in all groups of chronic and acute carbon tetrachloride intoxicated rats. Also, there were no changes in reticulocyte count, plasma iron and total iron binding capacity levels in chronic intoxicated animals. However, in acute carbon tetrachloride plasma iron and total iron-binding capacity were significantly elevated.

References

1. *Bingham, J.*, Blood **15**, 244 (1960). – 2. *Fukuoka, S. H.*, Acta Med. **45**, 821 (1954). – 3. *Gutman, A. P.*, Adv. in protein chem. **4**, 155 (1948). – 4. *Hall, C. A.*, J. Lab. Clin. Med. **48**, 395 (1956). – 5. *Hoffman, J., M. B. Himes, S. Lapan, R. Risziki, N. Post*, Arch. Pathol. **59**, 429 (1955). – 6. *King, E., L. D. Wooton*, In: Micro Analysis in Medical Biochemistry, P. 950 (London 1959). – 7. *LaMotta, R., H. Williams, H. Wastone*, Gastroenterol. **33**, 50 (1957). – 8. *Lieweliyn, O. P.:* Ann. Occup. Hyg. **9**, 199 (1966). – 9. *Loh, T. T., J. S. Juggi*, Aust. J. Exp. Biol. Med. Sci. **49**, 493 (1971). – 10. *Mangalik, V. S., R. M. L. Methrota, N. C. Nayak*, J. Path. Bact. **63**, 239 (1957). – 11. *Mazur, A., A. Carleton, A. Carlsen*, J. Biol. Chem., **236**, 1109 (1961). – 12. *McCollister, D. D., W. H. Beamer, G. J. Atchison, H. C. Spencer*, J. Pharmacol. **102**, (1951). – 13. *McCloskey, J. F., E. H. McGehce*, Arch. Pathol., **49**, 200 (1955). – 14. *Miale, J. B.*, Laboratory Medicine: Haemotology, 3rd ed. P. 1125 (St. Louis 1967). – 15. *Patty, F. D.*, Industrial Hygiene and Toxicology, Interscience (New York 1961). – 16. *Rammsey, W. N. M.*, Clin. Chem. Acta **2**, 214 (1957). – 17. *Reissmann, K. R., J. Boley, J. F. Christanson, M. H. Delp*, J. Lab. Clin. Med. **43**, 572 (1954). – 18. *Reissmann, K. R., M. R. Dietrich*, J. Clin. Invest. **35**, 580 (1956). – 19. *Robbins, B. H.*, J. Pharmacol. Exp. Therap. **37**, 203 (1929). – 20. *Sherlock, S.*, Diseases of the liver and biliary system, 4th edition, Blackwell (1960). – 21. *Suzuki, S.*, J. Lab. Pharmac. **17**, 393 (1967). – 22. *Wells, H. S.*, Exptl., Therp. **25**, 235 (1925). – 23. *Wong, J.*, J. Biol. Chem. **77**, 409 (1928). – 24. *Zinkel, J. G., R. M. Bush, C. E. Cornelins, R. A. Freed Land*, Res. Vet. Sci., **12**, 211 (1971).

Authors' address:

Dr. *E. A. El-Dessoukey*, National Research Centre,
Industrial Medicine, Dokki, Cairo (Egypt)