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Effect of drugs affecting microsomal enzymes on serum protein pattern and fat content of the liver

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With 2 tables

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In animals, pathological studies have demonstrated that the liver is the primary site of attack of the drug. The ease with which CCl_4 produces liver lesions and the precise histological control rendered this drug a useful tool for liver studies.

The importance of the activity of the microsomal enzymes in the CCl_4 induced liver injury has been previously emphasized. In some experiments where the activity was reduced the animals proved resistant to the poison (6, 25). By contrast the increase of the enzyme activity leads to increased sensitivity to CCl_4 (25).

However, we found that phenobarbitone which is known to be an inducer of microsomal enzyme, when given in repeated doses together with small doses of CCl_4 protected rats against the hepatotoxicity of CCl_4. Protection was evident from reduced activity of plasma glutamic-pyruvic transaminase and reduced liver fatty degeneration as demonstrated by histologic evaluations. Similar treatment with propionyl-promazine which is a microsomal enzyme inhibitor, together with CCl_4, failed to protect rats against CCl_4 induced hepatotoxicity (7).

The action of most hepatotoxic drugs which cause fatty liver is, in general, associated with inhibition of hepatic protein synthesis (2).

The aim of the present study is to evaluate the effect of phenobarbitone and propionyl-promazine on serum protein pattern and fat content of the liver in carbon-tetrachloride induced liver damage.

Material and methods

Male albino rats (Sprague Dawley strain) were used. The diet was an adequate one and was supplied ad libitum. The animals were divided into six groups. Ten animals were included in each group.

Control group, group treated with CCl_4 (0.1 ml/kg S.C.) diluted in the ratio of 1:1 with paraffin oil was administered daily for ten days. Phenobarbitone group (60 mg/kg i.p.), propionyl-promazine group (2 mg/kg i.m.) and two other groups administered daily, either phenobarbitone plus CCl_4 or propionyl-promazine plus CCl_4, for a period of 10 days. The animals were weighed before and after the experiment, and were sacrificed by light ether anesthesia. Blood samples were taken from the orbital plexus and serum was kept frozen for further analysis.

Total serum proteins was determined by the biuret method and electrophoretic separation of serum proteins by the method of *King* and *Wootton* (22).

Liver samples were taken for fat analysis. The fat content of the liver was determined according to *Folch* (9).

Results

Data describing the fat content of the liver and percentage change in body weight and serum total protein and its fractions in control and carbon-tetrachloride intoxicated rats under the influence of phenobarbitone and propionyl-promazine are represented in table 1 and 2.

Table 1. Effect of daily administration of phenobarbitone or propionyl-promazine together with S.C. injection of CCl_4 on fat content of the liver and body wt. change.

Experimental groups	Doses given daily for 10 days	Fat as % of wet liver wt.	P*	Body wt. changes %
1. Control	–	4.27 ± 0.34	0.005	+16.0
2. CCl_4	0.1 ml/kg s.c.	14.77 ± 1.06	–	– 5.5
3. Phenobarbitone	60 mg/kg i.p.	6.22 ± 0.54	0.05	– 3.5
4. Phenobarbitone + CCl_4	60 mg/kg i.p.	7.47 ± 0.49	0.05	– 6.0
5. Propionyl-promazine	2 mg/kg i.m.	4.29 ± 0.55	0.005	+13.0
6. Propionyl-promazine + CCl_4	2 mg/kg i.m.	12.99 ± 0.75	0.05	+31.0

* Experimental groups vs CCl_4

Our data revealed a marked increase of the fat content of the liver under the influence of carbon-tetrachloride.

However, when phenobarbitone was administered together with CCl_4 this treatment resulted in a significant reduction in the fat content of the liver.

Nevertheless, in comparison with the control experiments it was found that under the influence of phenobarbitone whether was administered alone or together with carbon-tetrachloride, the fat content of the liver was significantly higher than that of the control.

Propionyl-promazine when administered alone or together with CCl_4 has no effect on the fat content of the liver.

Conserning the body weight, it was found that there is a gain in the body weight of the control group, while there is a loss of body weight under the influence of CCl_4 phenobarbitone and phenobarbitone plus carbontetrachloride. However, there is also a body weight increase under the influence of propionyl-promazine whether it was administered alone or together with CCl_4.

Table 2 represents the changes in the total protein and its fractions. A state of hypoproteinemia and hypoalbuminemia may be observed in rats with severe conditions of carbon-tetrachloride intoxication, while the

Table 2. Serum protein pattern in control and studied groups (mean ± S.E.).

Groups	T. pr. g%	Alb g%	α-G g%	B-G g%	γ-G g%	T. G. g%	A/G ratio
Control	7.39	3.42	1.30	1.61	1.06	3.97	0.84
	±0.35	±0.09	±0.05	±0.06	±0.05	±0.19	±0.008
CCl_4	6.19	1.88	1.64	1.91	0.96	4.31	0.45
	±0.48	±0.06	±0.05	±0.06	±0.03	±0.18	±0.003
P	0.025	0.005	0.05	0.025	0.05	0.025	0.005
Phenobarbitone	6.62	2.46	1.35	1.65	1.16	4.11	0.59
	±0.39	±0.08	±0.05	±0.06	±0.04	±0.12	±0.004
P	0.05	0.05	0.15	0.15	0.15	0.10	0.025
Phenobarbitone + CCl_4	7.04	2.96	1.39	1.68	1.11	4.18	0.70
	±0.47	±0.08	±0.05	±0.06	±0.04	±0.13	±0.006
P	0.10	0.15	0.15	0.15	0.15	0.10	0.10
Propionyl-promazine	6.78	2.68	1.33	1.56	1.21	4.10	0.64
	±0.39	±0.07	±0.04	±0.05	±0.04	±0.15	±0.005
P	0.05	0.08	0.15	0.15	0.025	0.10	0.05
Propionyl-promazine + CCl_4	6.50	1.97	1.53	1.56	1.32	4.53	0.43
	±0.56	±0.09	±0.06	±0.06	±0.05	±0.16	±0.003
	0.05	0.005	0.10	0.15	0.025	0.025	0.005

globulin fraction increased. The A/G ratio was therefore significantly decreased.

The effect of phenobarbitone on serum proteins of normal rats, resulted in a significant decrease in total protein and in serum albumin, while the globulin fractions remained virtually unaltered. The A/G ratio was therefore significantly decreased.

However, when phenobarbitone was administered together with carbon-tetrachloride, there was no statistically significant difference in the percentage of the different protein fractions nor in the A/G ratio as compared with the control group.

In the case of propionyl-promazine when administered to control rats it resulted in a significant decrease in total protein and increase in gamma globulin. The A/G ratio was significantly decreased.

Under the influence of propionyl-promazine plus carbon-tetrachloride there was a state of hypoproteinemia and hypoalbuminemia and a significant increase in gamma globulin with consequent decrease in A/G ratio.

Discussion

The hepatic lipid level is affected by a number of factors. These include the release of FFA from adipose tissue, uptake of FFA by the liver, hepatic FFA metabolism (synthesis, oxidation, incorporation into triglycerides, and phospholipids) and release of lipid from the liver as lipoprotein.

Accumulation of abnormal amounts of lipid in the liver could conceivably result from a change in the rate of one or more of these steps.

Chemical investigations revealed that the rise in liver lipid following administration of CCl_4 to rats was due to increased liver triglyceride (5, 33).

Maling et al. (11, 24), were the first to demonstrate that after carbon-tetrachloride poisoning there is a marked reduction in transfer of triglycerides from liver to plasma.

The demonstration that a hepatic triglyceride secretory mechanism was blocked shortly after carbon-tetrachloride poisoning established the pathophysiological basis of carbon-tetrachloride fatty liver (29).

In CCl_4 poisoning, early morphological changes have been described in the endoplasmic reticulum of the liver cell (3, 27). At the same time the endoplasmic reticulum was damaged both enzymatically (26, 30) and structurally (28). Simultaneously liver triglycerides were rising rapidly (33).

Recknagel and *Lombardi* (30) found evidence of damage to the endoplasmic reticulum 2 to 3 hr after CCl_4 poisoning coinciding in time with the highest CCl_4 concentration in the liver and with the beginning of fat accumulation. They considered that the endoplasmic reticulum had a role in lipid secretion and postulated that in CCl_4 poisoning the fatty liver resulted from an accumulation of the triglycerides normally secreted by the liver.

Inhibition of the secretion of T.G. synthesized in the liver could be due either to an impairment of the triglyceride secreting mechanism in the livers of poisoned animals (30) *Recknagel* and *Lombardy* (30) and/or to a reduced synthesis of the protein necessary for the formation of lipoprotein, the vehicle which transports triglycerides from the liver (31).

Depressed secretion of T.G. from the liver following CCl_4 was suggested by *Seakins* et al. (34) to be related to depressed carrier protein synthesis.

Henshaw et al. (16) have suggested that endoplasmic membranes may play a role in the protein synthesizing maschinery of microsomes. (Microsomes attached to the membrane produce more proteins than free ribosomes.)

Our data revealed depressed proteins synthesis in the carbon-tetrachloride intoxicated group. A loss in body weight together with a decline in the levels of serum total protein and albumin is shown in table (1, 2).

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 which are responsible for the synthesis of plasma proteins. Another contributing factor that may affect protein synthesis and body weight 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 (35).

However, the increase in body weight noticed in case of propionyl-promazine especially when administered together with carbon-tetrachloride which is accompanied by hypoproteinemia, hypoalbuminemia and fatty

hyperalbuminemia liver may be explained due to body oedema. It was suggested that administration of propionyl-promazine cause water and electrolye retention (10).

An increase in the level of alpha globulins in carbon-tetrachloride induced liver damage in rats was found. Such increase in alpha globulins encountered in our intoxicated rats, agrees with data previously reported by *Gutman* (15) 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 carbon-tetrachloride treated group corresponds to the previous work of *Fukuoka* (12). Such increase may be attributed to the increased synthesis of beta globulin in the liver to compensate the lowering of serum albumin. Also *Zinkel* et al. (37) reported an increase in the beta globulin during carbon-tetrachloride intoxication.

The observed decrease in the A/G ratio in CCl_4 treated group is caused by the decrease of albumin as well as the increase in the total globulin levels.

Istvan et al. (18) found that a single i.p. injection of 1 ml CCl_4/100 g to rats induced hypoproteinemia and decreased the blood albumin to globulin ratio.

Kidney damage may also play a role in the hypoproteinemia observed following CCl_4 poisoning. It was found that i.p. administration of 0.1 ml CCl_4/100 g to rats induced histological kidney damage, hypoproteinemia and proteinuria. The urinary proteins were mostly albumin (19).

Interference with hepatic protein synthesis could readily lead to the development of a fatty liver (17). *Farber* (8) suggested that protein changes precede the fatty liver by a few hours. *Guentera* et al. (14) found that protein synthesis of rat liver microsomes was inhibited 30 min after i.p. injection of 0.2 ml CCl_4/100 g.

In the present work the liver fat was markedly increased in CCl_4 treated group accompanied by serum hypoproteinemia and hypoalbuminemia. Thus in case of CCl_4 there is a direct evidence implicating impairment of protein synthesis accompanying fatty liver.

The finding that the fat content of the liver increased under the influence of phenobarbitone, is in accord to the previous work of *Sorrell* et al. (36), who found that administration of phenobarbital to rats produced elevated levels of liver TG, phospholipid and total cholesterol. They showed that choline and fat content of the diet are factors influencing the amount of TG accumulating in the liver as a result of phenobarbital administration.

Table 1 shows that the fat content of the liver was high when CCl_4 administration was combined with propionyl-promazine. However, liver fat decreased significantly when CCl_4 administration was combined with phenobarbitone. This shows that phenobarbitone had a protective effect on the hepatotoxicity produced by carbon-tetrachloride while propionyl-promazine had not. This is also shown from the serum protein pattern (table 2).

Thus a state of hypoproteinemia and hypoalbuminemia was observed in rats with carbon-tetrachloride intoxication while the globulin fractions increased with consequent decrease in A/G ratio.

However, when phenobarbitone was administered together with CCl_4, there was no statistically significant difference in the percentage of the different protein fractions nor in the A/G ratio as compared with the control group. While in case of propionyl-promazine plus CCl_4 there was no difference in the protein pattern nor in the A/G ratio from carbon-tetrachloride group.

Kato et al. (21) have shown that pretreatment with phenobarbitone in rats stimulates the incorporation of labelled leucine into microsomal proteins in vivo. This mechanism of action of phenobarbitone is just physiologically opposite to the mechanism of the toxic action of CCl_4 in rats (31). Phenobarbitone has also been shown to increase the incorporation of arginine-leucine, cysteine, phenylalanine and vline into microsomal proteins.

Thus the protective action of phenobarbitone on carbon-tetrachloride induced hepatotoxicity in rats may be due to an antagonistic action of phenobarbitone in carbon-tetrachloride induced depressed incorporation of amino acids in microsomal proteins.

Gadgil et al. (13) suggested that the main protective action of phenobarbitone on carbon-tetrachloride hepatotoxicity may be due to its effect on microsomes or ribosomes attached to the smooth surfaced endoplasmic reticulum.

Koff et al. (23) found the phenobarbital inhibit the ethanol induced triglyceride accumulation and that this is accompanied by alterations of the endoplasmic reticulum of the hepatocyte.

The effects of microsomal enzyme inducers on hepatic function in mice and rats were studied when these drugs were given before or after poisoning with CCl_4. If daily administration of Na-phenobarbital was begun 12–18 hr after CCl_4 intoxication, the 20 min bromosulfophthalein (BSP) retention value returned to normal in 3 to 4 days. However, Na-phenobarbital pretreatment increased mortality after CCl_4 (1).

The present results show that phenobarbitone, although a potent microsomal enzyme inducer, protected rats against the hepatotoxicity of CCl_4, when given in ten repeated doses together with small doses of CCl_4.

Brodie et al. (4) have demonstrated that the enzymes responsible for the metabolism of the barbiturates are located within the endoplasmic reticulum (microsomes) of the liver cells.

Kato et al. (20) and *Rogers* et al. (32) found that the metabolism of barbiturates is inhibited by the simultaneous administration of other drugs which compete with the barbiturates for the same drug-metabolizing enzymes. Subsequent to this initial phase of competitive inhibition there may follow, usually after an interval of 12 hours or so, a period of increased metabolic activity due to the induction of the drug-metabolizing enzymes.

Thus inhibition and induction of microsomal enzymes are therefore often relative terms determined by the time interval between pretreatment of the animal with other drugs, and the administration of the barbiturate.

Summary

The influence of phenobarbitone and propionyl-promazine given in ten repeated doses together with small doses of CCl_4 on serum protein pattern and fat content of the liver was investigated in albino rats.

The data revealed a marked increase of the fat content of the liver under the influence of CCl_4. However, when phenobarbitone was administered together with CCl_4 it resulted in a significant reduction in the fat content of the liver.

Nevertheless, in comparison with the control experiments it was found that under the influence of phenobarbitone whether it was administered alone or together with CCl_4, the fat content of the liver was significantly higher than that of the control.

Concerning the changes in the total protein and its fractions, a state of hypoproteinemia and hypoalbuminemia was observed in rats with severe CCl_4 intoxication. While the globulin fractions increased, the A/G ratio was therefore significantly decreased.

However, when phenobarbitone was administered together with CCl_4 there was no statistically significant difference in the percentage of the different protein fractions nor in the A/G ratio as compared with the control group.

Propionyl-promazine when was administered alone or together with CCl_4 has no effect on the fat content of the liver and serum protein pattern.

It is concluded that under the present experimental conditions, the main protective effect of phenobarbitone seems to be due to an antagonistic action of phenobarbitone in CCl_4 induced decomposition at the level of the endoplasmic reticulum.

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