Biochemical Pharmacology, Vol. 19, pp. 1463–1469. Pergamon Press. 1970. Printed in Great Britain

CHANGES IN THE SERUM CONCENTRATION OF CHOLESTEROL, TRIGLYCERIDES AND PHOSPHOLIPIDS IN THE MOUSE AND RAT AFTER ADMINISTRATION OF EITHER CHLORCYCLIZINE OR PHENOBARBITAL*

R. A. SALVADOR, C. ATKINS, S. HABER and A. H. CONNEY

The Wellcome Research Laboratories, Burroughs Wellcome & Co. (U.S.A.) Inc., Tuckahoe, N.Y. 10707, U.S.A.

(*Received* 29 *July* 1969; *accepted* 29 *September* 1969)

Abstract—The administration of chlorcyclizine or phenobarbital reduced the serum concentration of cholesterol, triglycerides and phospholipids in the male and female mouse, and chlorcyclizine was more potent than phenobarbital. The reductions caused by chlorcyclizine administration in the mouse were not always associated with an accumulation of cholesterol or triglycerides in liver. Chlorcyclizine had little or no effect on the serum concentration of cholesterol and phospholipids in the rat, but the serum concentration of triglycerides was reduced markedly. The reduction of serum triglycerides in the chlorcyclizine-treated rat was not correlated with either an increase in liver weight or an increase in the concentration of triglycerides in liver.

CHLORCYCLIZINE and a number of other diarylalkylpiperazines have been reported to lower significantly the serum concentration of cholesterol in the mouse.[1–3] The reduction in the concentration of serum cholesterol in the chlorcyclizine-treated mouse was accompanied by an increase in liver weight and in the liver concentration of cholesterol. It was suggested that the increase in the cholesterol content of liver was the result of either a redistribution of cholesterol or an increase in cholesterol biosynthesis.[1, 2] This report describes the effect of chlorcyclizine, several chlorcyclizine analogs and phenobarbital on the serum and liver concentration of cholesterol (CL)† and triglycerides (TG) in the mouse. It also describes the effect of chlorcyclizine on the same parameters in the rat.

METHODS

Mice of the CF-1 strain‡ and rats of the Long–Evans and Sprague–Dawley (CFE) strain were fed Wayne Lab Blox meal§ *ad lib*. Each drug was mixed into the Lab Blox meal mechanically and the drug-containing diet was fed to the animals for 9 days. Each diet was fed to groups of twenty-five mice or 8–12 rats. The mice and rats were fasted overnight on the ninth day and the animals were killed the next morning. At

* A preliminary report of these results was presented at the Third International Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy (September 1968).

† The following abbreviations are used: CL, cholesterol; TG, triglyceride(s); PL, phospholipid(s),

‡ CF-1 mice and Sprague–Dawley rats were obtained from Carworth Farms, Inc., New York, N. Y.; Long–Evans rats were obtained from Blue Spruce Farms, Altamont, N. Y.

§ Wayne Lab Blox meal mouse or rat diet in the unpelleted form was obtained from Allied Mills Inc., Chicago, Ill.

that time, the total body and liver weight was determined for each animal. Five pooled serums were obtained from each group of twenty-five mice by pooling the blood of five randomly selected mice for each serum sample. The serum of each rat was analyzed separately. Each serum sample was analyzed for CL, TG and phospholipids (PL). Ten per cent liver homogenates were prepared with saline using all glass homogenizers, and the homogenates were analyzed for their content of CL and TG. A 0·5-ml portion or less of serum or liver homogenate was shaken mechanically for 10 min with enough isopropanol to give a final volume of 10 ml. This extract was filtered and total CL was determined in the filtrate using the Technicon Autoanalyzer procedure N-24a. Phospholipids were removed from a portion of the same filtrate by the addition of Zeolite or Florisil* and the TG content of the filtrate was determined by the procedure of Kessler and Lederer.[4] Using this procedure, the same TG concentrations were obtained when either Zeolite or Florisil was used to remove PL. The PL content of 0·1 ml serum was determined by the method of Zilversmit and Davis.[5] The data were analyzed statistically using the analysis of variance method in conjunction with Duncan's[6] multiple range test.

RESULTS

Effect of chlorcyclizine and phenobarbital on the serum concentration of cholesterol, triglycerides and phospholipids in the mouse. Chlorcyclizine and phenobarbital caused a significant reduction in the serum concentration of CL, TG and PL in the male mouse (Table 1). Serum CL was reduced 48, 45 and 30 per cent, respectively, when 0·08, 0·04 or 0·01 per cent chlorcyclizine was fed in the diet, while 0·05 per cent phenobarbital in the diet reduced serum CL by 19 per cent. The concentration of TG in the serum

TABLE 1. EFFECT OF CHLORCYCLIZINE AND PHENOBARBITAL ON THE SERUM AND LIVER CONCENTRATION OF CHOLESTEROL (CL), TRIGLYCERIDES (TG) AND PHOSPHOLIPIDS (PL) IN THE MALE MOUSE*

Addition to diet	Body wt. (g ± S. E.)	Liver wt. (g ± S. E.)	Serum concentration (mg/100 ml ± S. E.)			Liver concentration (mg/g ± S. E.)	
			CL	TG	PL	CL	TG
None	22 ± 0·4	1·0 ± 0·02	155 ± 3	117 ± 6	320 ± 11	8·4 ± 0·4	22 ± 3
Chlorcyclizine (0·08%)	19 ± 0·4†	1·5 ± 0·05†	80 ± 5†	46 ± 10†	158 ± 16†	8·7 ± 0·6	19 ± 3
Chlorcyclizine (0·04%)	20 ± 0·3†	1·3 ± 0·03†	86 ± 6†	52 ± 5†	180 ± 15†	9·2 ± 0·5	32 ± 6
Chlorcyclizine (0·01%)	21 ± 0·3	1·2 ± 0·03†	109 ± 6†	76 ± 6†	227 ± 9†	7·4 ± 0·4	26 ± 6
Phenobarbital (0·05%)	20 ± 0·4†	1·1 ± 0·03	126 ± 7†	85 ± 13‡	243 ± 3†	7·1 ± 0·4	17 ± 5

* Groups of twenty-five mice were fed chlorcyclizine HCl or phenobarbital for 9 days. The mice were killed on the tenth day after an overnight fast. Five serum samples from each group were analyzed for CL, TG and PL. Each serum sample was obtained by pooling the blood of five mice. Ten livers were selected randomly from each group for the analysis of CL and TG. The results were compared statistically using the analysis of variance in conjunction with Duncan's[6] multiple range test.
† $P < 0.01$.
‡ $P < 0.05$.

* Zeolite was obtained from W. A. Taylor Company, Baltimore, Md.; Florisil was obtained from Fisher Scientific Company.

was reduced 61, 55 and 35 per cent, respectively, with 0·08, 0·04 and 0·01 per cent chlorcyclizine in the diet, while 0·05 per cent phenobarbital in the diet caused a 27 per cent reduction in serum TG. Serum PL was reduced 50, 44 and 29 per cent, respectively, when 0·08, 0·04 and 0·01 per cent chlorcyclizine was fed in the diet, while 0·05 per cent phenobarbital in the diet reduced serum PL by 24 per cent. The liver concentration of CL and TG was not affected by the administration of chlorcyclizine or phenobarbital. A 10 per cent reduction in body weight occurred in mice fed 0·08 and 0·04 per cent chlorcyclizine, but no change in body weight occurred in mice fed 0·01 per cent chlorcyclizine. A 9 per cent decrease in body weight occurred in mice fed 0·05 per cent phenobarbital in the diet. Liver weight increased approximately 50, 30 and 20 per cent in mice that were fed 0·08, 0·04 and 0·01 per cent chlorcyclizine in the diet.

The effect of chlorcyclizine and phenobarbital on the serum concentration of CL, TG and PL in the female mouse is shown in Table 2. The administration of 0·025 per

TABLE 2. EFFECT OF CHLORCYCLIZINE AND PHENOBARBITAL ON THE SERUM AND LIVER CONCENTRATION OF CHOLESTEROL (CL), TRIGLYCERIDES (TG) AND PHOSPHOLIPIDS (PL) IN THE FEMALE MOUSE*

Addition to diet	Body wt. (g ± S. E.)	Liver wt. (g ± S. E.)	Serum concentration (mg/100 ml ± S. E.)			Liver concentration (mg/g ± S. E.)	
			CL	TG	PL	CL	TG
None	16 ± 0·1	0·8 ± 0·01	134 ± 4	49 ± 4	264 ± 8	4·6 ± 0·1	25 ± 4
Chlorcyclizine (0·025%)	16 ± 0·1	1·0 ± 0·02†	77 ± 4†	13 ± 1†	149 ± 8†	6·3 ± 0·2†	41 ± 5
Phenobarbital (0·05%)	16 ± 0·1	1·0 ± 0·05†	110 ± 7†	28 ± 2†	200 ± 6†	5·8 ± 0·2†	39 ± 5
Phenobarbital (0·01%)	16 ± 0·1	0·9 ± 0·02	119 ± 3	42 ± 3		5·1 ± 0·2	50 ± 8†

* The experimental procedure is given in Table 1 and in Methods. The statistical method is described in Table 1.
† $P < 0·01$.

cent chlorcyclizine in the diet resulted in a significant reduction in serum CL (42%), TG (73%) and PL (43%). With 0·05 per cent phenobarbital in the diet, there was a smaller but significant reduction in the serum concentration of CL (18%), TG (42%) and PL (24%). The concentration of CL and TG was not reduced significantly in the serum of mice fed 0·01 per cent phenobarbital in the diet. The liver concentration of CL increased 37 per cent in female mice fed 0·025 per cent chlorcyclizine and 26 per cent in female mice fed 0·05 per cent phenobarbital. The mean TG concentration in the liver was also increased in mice fed chlorcyclizine or phenobarbital. The body weight of female mice described in Table 2 was not affected by drug administration. Liver weight increased 25 per cent in mice fed 0·025 per cent chlorcyclizine or 0·05 per cent phenobarbital.

Effect of chlorcyclizine on the serum concentration of cholesterol, triglycerides and phospholipids in the rat. The effect of various doses of chlorcyclizine on the serum concentration of CL, TG and PL in the male rat of the Long–Evans strain is shown in Table 3. The serum concentration of cholesterol was reduced only 11–14 per cent in

TABLE 3. EFFECT OF CHLORCYCLIZINE ON THE SERUM AND LIVER CONCENTRATION OF CHOLESTEROL (CL), TRIGLYCERIDES (TG) AND PHOSPHOLIPIDS (PL) IN THE LONG–EVANS MALE RAT*

Chlorcyclizine in diet (%)	Body wt. (g ± S. E.)	Liver wt. (g ± S. E.)	Serum concentration (mg/100 ml ± S. E.)			Liver concentration (mg/g ± S.E.)	
			CL	TG	PL	CL	TG
0	189 ± 5	6·5 ± 0·2	71 ± 2	56 ± 4	144 ± 7	7·6 ± 0·1	2·7 ± 0·1
0·08	163 ± 4†	7·8 ± 0·2†	61 ± 3†	19 ± 1†	123 ± 8‡	9·4 ± 0·3†	5·4 ± 0·8†
0·04	185 ± 4	8·2 ± 0·2†	63 ± 2†	30 ± 1†	135 ± 5	8·8 ± 0·4†	4·1 ± 0·2‡
0·01	191 ± 5	6·8 ± 0·3	71 ± 1	40 ± 2†	154 ± 5	7·5 ± 0·1	2·7 ± 0·1

* Groups of twelve rats were fed drug for 9 days. The rats were killed on the tenth day after an overnight fast and the serum and liver of each rat were analyzed. The statistical method is described in Table 1.
† $P < 0.01$.
‡ $P < 0.05$.

rats fed 0·08 or 0·04 per cent chlorcyclizine in the diet for 9 days, and no effect was observed in rats fed 0·01 per cent chlorcyclizine. A reduction in the serum concentration of PL occurred only in rats fed 0·08 per cent chlorcyclizine. Unlike the results obtained in the mouse, the concentration of CL and PL in rat serum was not always reduced when chlorcyclizine was administered. In a second experiment, neither the serum concentration of CL or PL was reduced as a result of feeding 0·08, 0·04 or 0·01 per cent chlorcyclizine in the diet for 9 days. Interestingly, the serum concentration of TG was reduced markedly with all doses of chlorcyclizine. The liver concentration of TG was unchanged in rats given 0·01 per cent chlorcyclizine in the diet, but increased significantly in rats fed 0·08 or 0·04 per cent chlorcyclizine. It is of particular interest that the reduction in serum TG caused by feeding 0·01 per cent chlorcyclizine was not associated with an increase in the liver concentration of TG or with a significant increase in liver weight. The liver concentration of CL increased consistently in rats given 0·08 per cent chlorcyclizine in the diet, while this increase was less consistent in rats fed 0·04 per cent chlorcyclizine.

Chlorcyclizine was fed to adult male rats of the Sprague–Dawley strain (Table 4).

TABLE 4. EFFECT OF CHLORCYCLIZINE ON THE SERUM AND LIVER CONCENTRATION OF CHOLESTEROL (CL), TRIGLYCERIDES (TG) AND PHOSPHOLIPIDS (PL) IN THE SPRAGUE–DAWLEY MALE RAT*

Chlorcyclizine in diet (%)	Body wt. (g ± S. E.)	Liver wt. (g ± S. E.)	Serum concentration (mg/100 ml ± S. E.)			Liver concentration (mg/g ± S. E.)	
			CL	TG	PL	CL	TG
0	154 ± 6	5·3 ± 0·2	81 ± 3	77 ± 9	189 ± 4	2·8 ± 0·1	5·6 ± 0·7
0·08	153 ± 2	7·5 ± 0·2†	80 ± 5	29 ± 7†	159 ± 8†	3·5 ± 0·2†	12·7 ± 1·7†
0·04	147 ± 4	6·2 ± 0·2‡	80 ± 4	43 ± 7†	159 ± 10†	2·7 ± 0·1	7·9 ± 0·6
0·01	155 ± 5	5·5 ± 0·3	78 ± 4	46 ± 5†	172 ± 5	2·5 ± 0·1‡	5·8 ± 1·0

* The experimental procedure is given in Table 3 and in Methods. Eight or nine rats were used in each group. The statistical method is described in Table 1.
† $P < 0.01$.
‡ $P < 0.05$.

The serum concentration of CL was not affected by chlorcyclizine administration, while the concentration of PL in serum was slightly reduced in rats fed 0·08 and 0·04 per cent chlorcyclizine in the diet. The concentration of serum TG was reduced markedly with all doses of chlorcyclizine and the reduction in serum TG in rats fed 0·01 per cent chlorcyclizine was not associated with either a significant increase in liver weight or a significant increase in the liver concentration of CL or TG. A significant increase in the liver concentration of CL and TG occurred only in rats fed 0·08 per cent chlorcyclizine. There was no reduction in the serum concentration of CL in rats fed 0·08 per cent chlorcyclizine, although liver weight and the concentration of CL in the liver increased significantly.

Reduction of the serum concentration of cholesterol and triglycerides in the mouse by analogs of chlorcyclizine. Several chlorcyclizine analogs were compared with chlorcyclizine for their effect on the serum and liver concentration of CL and TG in the female mouse (Table 5). Each drug was fed in the diet to twenty-five mice for 9 days

TABLE 5. COMPARISON OF THE EFFECT OF CHLORCYCLIZINE AND SEVERAL CHLORCYCLIZINE ANALOGS ON THE SERUM AND LIVER CONCENTRATION OF CHOLESTEROL (CL) AND TRIGLYCERIDES (TG) IN THE FEMALE MOUSE*

Addition to diet	Body wt. (g ± S. E.)	Liver wt. (g ± S. E.)	Serum concentration (mg/100 ml ± S. E.)		Liver concentration (mg/g ± S. E.)	
			CL	TG	CL	TG
None	19·3 ± 0·2	0·95 ± 0·02	139 ± 8	101 ± 8	5·9 ± 0·1	40 ± 19
Chlorcyclizine	18·4 ± 0·3	1·05 ± 0·03†	116 ± 12‡	67 ± 10‡	7·1 ± 0·3†	30 ± 13
Norchlorcyclizine	19·6 ± 0·2	1·05 ± 0·02†	93 ± 19†	47 ± 4†	6·5 ± 0·2	53 ± 11
N-ethyl-N^1-benzhydrylpiperazine	18·3 ± 0·2	0·92 ± 0·03	119 ± 6‡	55 ± 6†	6·2 ± 0·2	60 ± 4
p-(N^1-methylpiperazine)benzophenone	18·4 ± 0·3	0·93 ± 0·02	145 ± 6	96 ± 9	5·9 ± 0·1	23 ± 4
2-(N^1-methylpiperazino)benzophenone	18·7 ± 0·3	0·97 ± 0·03	145 ± 3	85 ± 8	5·7 ± 0·2	18 ± 5
N,N^1-dibenzoylpiperazine	18·1 ± 0·4	0·90 ± 0·03	157 ± 3	84 ± 5	5·7 ± 0·2	23 ± 5
N-methyl-N^1-(9-fluorenyl) piperazine	19·5 ± 0·4	0·91 ± 0·02	130 ± 6	86 ± 5	6·2 ± 0·1	46 ± 10

* Chlorcyclizine and its analogs were administered as the hydrochloride salts. The experimental procedure is given in Table 1 and in Methods. The statistical method is described in Table 1.
† $P < 0·01$.
‡ $P < 0·05$.

at a concentration of 0·01 per cent. A significant reduction in the serum concentration of CL and TG occurred with the administration of chlorcyclizine, norchlorcyclizine and *N*-ethyl-*N'*-benzhydrylpiperazine. The serum concentration of CL and TG was not reduced by the administration of several chlorcyclizine analogs which did not contain the benzhydryl portion of the molecule. These compounds included *p*-(*N'*-methylpiperazine) benzophenone, 2-(*N'*-methylpiperazino) benzophenone, *N,N'*-dibenzoylpiperazine and *N*-methyl-*N'*-(9-fluorenyl) piperazine. Liver weight increased 10–15 per cent in mice fed chlorcyclizine or norchlorcyclizine, but not in mice fed *N*-ethyl-*N'*-benzhydryl piperazine. Body weight was not affected by drug administration. A 20 per cent increase in liver CL occurred in mice given chlorcyclizine, but no

change in the concentration of liver cholesterol occurred after the administration of norchlorcyclizine or *N*-ethyl-*N'*-benzhydrylpiperazine. The reduction in serum TG which occurred after the administration of chlorcyclizine, norchlorcyclizine and *N*-ethyl-*N'*-benzhydrylpiperazine was not accompanied by an increase in the concentration of TG in the liver.

DISCUSSION

Chlorcyclizine has been reported to lower serum CL in the male mouse and rat.[1] Our results in the male and female mouse show that in addition to lowering serum CL, chlorcyclizine lowers the serum concentration of TG and PL. Phenobarbital also lowers significantly the serum concentration of CL, TG and PL in the male and female mouse, but this drug is less potent than chlorcyclizine. The CL and TG content of liver increased in female mice fed either chlorcyclizine or phenobarbital, but this effect was not found in male mice. Two analogs of chlorcyclizine (norchlorcyclizine and *N*-ethyl-*N'*-benzhydrylpiperazine) also caused a significant reduction in the serum concentration of CL and TG of the female mouse without causing an accumulation of these substances in liver. The results indicate that the lowering of serum CL and TG in the mouse by chlorcyclizine and its analogs is probably not related to an accumulation of these substances in liver.

Administration of chlorcyclizine in the diet of either the Long–Evans or Sprague–Dawley male rat caused only a small reduction in the serum concentration of CL or PL, but serum TG were reduced significantly. Others have reported that phenobarbital has no effect on the serum concentration of cholesterol in the rat.[7] An accumulation of CL and TG occurred in the liver of the male rat fed either 0·08 or 0·04 per cent chlorcyclizine in the diet, and liver weight increased significantly. There was no increase in the liver concentration of CL or TG when 0·01 per cent chlorcyclizine was fed in the diet, but a significant reduction of serum TG occurred. No increase in liver weight was observed in these animals. The results indicate that the chlorcyclizine-induced reduction of serum TG in the rat can occur in the absence of either an accumulation of TG in liver or an increase in liver weight.

Chronic administration of chlorcyclizine or phenobarbital increases the levels of enzymes in the endoplasmic reticulum of liver cells which metabolize drugs, steroid hormones and other normal body constituents,[8] whereas a single injection of chlorcyclizine into rats inhibits microsomal hydroxylation reactions for several hours.[9] It has been shown that the chronic administration of phenobarbital to the rat stimulates the formation of the enzyme system in liver microsomes that is required for the 7α-hydroxylation of cholesterol, an early step in the conversion of cholesterol to bile acids.[10] The reduction in the serum concentration of CL in the chlorcyclizine- or phenobarbital-treated mouse may be related to the induction of enzymes in the endoplasmic reticulum concerned with the metabolism of CL or the inhibition of microsomal enzymes involved in the synthesis of cholesterol. The ability of chlorcyclizine and phenobarbital to alter the activities of enzymes in the endoplasmic reticulum of liver cells is of particular interest, since this is the site of formation and secretion of serum lipoprotein. The alpha- and beta-lipoproteins of mouse serum were separated by paper electrophoresis, stained and quantitated with a densitometer. The results indicate that both alpha- and beta-lipoproteins were reduced markedly in chlorcyclizine-treated mice.

Acknowledgement—The authors gratefully acknowledge the assistance of Mr. R. Gauch and Mr. N. Kaufman with the statistical method.

REFERENCES

1. J. L. SCHMIDT and D. L. MARTIN, *Toxic. appl. Pharmac.* **7**, 257 (1965).
2. J. W. BARNHART and J. A. SEFRANKA, *Life Sci.* **5**, 871 (1966).
3. H. B. WRIGHT and D. L. MARTIN, *J. med. Chem.* **11**, 390 (1968).
4. G. KESSLER and H. LEDERER, *Technicon Symposium* (1965).
5. D. B. ZILVERSMIT and A. K. DAVIS, *J. Lab. clin. Med.* **35**, 155 (1950).
6. D. B. DUNCAN, *Biometrics* **11**, 1 (1955).
7. C. MITOMA, D. YASUDA, J. S. TAGG, S. E. NEUBAUER, F. J. CALDERONI and M. TANABE, *Biochem. Pharmac.* **17**, 1377 (1968).
8. A. H. CONNEY, *Pharmac. Rev.* **19**, 317 (1967).
9. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmac.* **13**, 69 (1964).
10. S. SHEFER, S. HAUSER and E. H. MOSBACH, *J. Lipid Res.* **9**, 328 (1968).