LACK OF ADRENOCORTICAL HORMONE-LIKE EFFECTS OF CHLORMADINONE ACETATE IN GUINEA PIGS

Toshi HORIUCHI, Masao MAKINO, Takaharu NAKAYAMA, Shuji MASUDA,
Kiyoko ITO, Hiroyoshi KUBOAKI, Hiroo TAKAHASHI
and Minoru R. SUZUKI

Pharmacological Research Department, Teikoku Hormone Mfg. Co., Ltd.
1, 604 Shimosakunobe, Takatsu-ku, Kawasaki 231, Japan

Received March 5, 1977

Summary.....Treatment with 0.01, 0.1 and 1.0% chlormadinone acetate (CMA) contained diets for 33 days to guinea pigs showed no signs of the depression in adrenal, thymus and leucocyte count and no elevation of serum cholesterol. By the treatment with 0.01 and 0.1% cortisol contained diets, however, the atrophy of adrenal and thymic cortex, hypercholesterolemia and the enlargement of liver were noted in this species. It was therefore concluded that CMA had no cortisone-like properties in guinea pigs even at the excessive doses.

INTRODUCTION

Chlormadinone acetate (17 α -acetoxy-6-chloro-pregna-4, 6-diene-3, 20-dione, CMA) is an orally active, progestational and anti-androgenic agent to laboratory animals (Brennan and Kraay, 1963; Dörner et al., 1972) and man (Dominguez et al., 1962; Geller et al., 1967). The inhibitory effects of some of the synthetic progestogens on adrenal and their adrenocortical hormone-like actions have been established since Glenn et al. (1959) described these properties of medroxyprogesterone acetate in rats. CMA was also demonstrated to cause the adrenal atrophy and cortisone-like effects in rats (Brennan and Kraay, 1963; Givner and Rochefort, 1972), dogs (Usui et al., 1977) and rabbits (Suzuki et al., 1977). Its action on adrenal in human is not well established. Reisert and Mühlen (1969) reported that daily dose of 30 mg CMA caused a slight impairment of adrenal function in volunteers. In a clinical report, however, daily dose of 100 mg CMA for 1 to 4 months showed no signs of adrenocortical suppression in patients with benign prostatic hyperplasia or prostatic cancer (Geller et al., 1967). In the present work, the effect of CMA on adrenal was examined in guinea pigs which secreted cortisol as a major component of glucocorticoids as well as man.

MATERIALS AND METHODS

Male and female guinea pigs of the Hartley strain (Matsumoto Exper. Animal Lab.), weighing 400-600g, were used. CMA and cortisol were given in the diet because of its difficulty to administer orally with a gastric tube in this species. The diets (CG3: CLEA JAPAN) contained 0.01, 0.1 and 1.0% CMA and 0.01 and 0.1% cortisol were fed for

33 days to 5 groups of 7–8 animals. Feed and water were freely available. The daily intakes of the drugs were estimated approximately as 4–6, 40–60 and 400–600 mg/kg in CMA fed groups and 4–6 and 40–60 mg/kg in cortisol fed groups, respectively. On completion of the medicated period, animals were anesthetized with sodium pentobarbital and blood was taken from abdominal artery. Red and white cell counts (Coulter Electronics Model ZBI) and differential leucocyte count (Giemsa's staining) were determined. Serum levels of cortisol (radioimmunoassay) and total cholesterol (method of Zak-Henly) were also estimated. Some of the visceral organs were removed and weighed. The thymic weights could not be weighed because of its difficulty to cut off from the adipose tissue surrounding it. Small portions of each organ were fixed in 10% buffered formalin, sectioned and stained with hematoxylin and eosin for histopathological examinations.

RESULTS AND DISCUSSION

Some animals fed CMA contained diet at the concentration of 1.0% showed a refusal of feeding and became moribund and 3 males and 4 females died during 11– to 17-day feeding period. Remaining 3 females were sacrificed on day 17. One female at the dose level of 0.1% of cortisol also died on day 17 and its autopsy finding revealed an infectious disease in the lung.

Weights of adrenal, liver, seminal vesicle and prostate Absolute and relative weights of adrenal, liver, seminal vesicle and prostate are presented in Table 1. In CMA fed groups, relative adrenal weights were significantly increased at the dose levels of 0.1 and/or 1.0% in each sex. Dietary CMA at the level of 0.1% caused a slight, but significant increase in weights of liver in females. The weights of seminal vesicle and prostate were significantly reduced at the dose levels of 0.1 and/or 1.0% of CMA in males. By the treatment with cortisol, absolute adrenal weights were significantly reduced in males but not in females. The weights of liver showed a dose-related increase in each sex treated with cortisol. Seminal vesicle and prostate showed no significant changes in cortisol treated groups.

Serum levels of cortisol and total cholesterol The serum levels of cortisol revealed a significant increase in males at the concentration of 1.0% of CMA as indicated in Table 2. The levels of total cholesterol showed a slight, but significant reduction at the dose level of 0.1% of CMA in males. In cortisol fed groups, a significant elevation of total cholesterol was noted at the levels of 0.01% in males and 0.01 and 0.1% in females.

Red and white cell counts and differential leucocyte count Dietary CMA caused no significant changes in red and white cell counts and differential leucocyte count as indicated in Table 3. By the treatment with cortisol, however, a significant increase of red cell counts was noted in all groups. Their leucocyte counts showed no significant change, but percentages of the lymphocytes were significantly depressed and the neutrophils were increased at the dose level of 0.1% of cortisol in each sex.

Histopathological findings By the treatment with CMA, the adrenal showed a

Chlormadinone and cortisol

Table 1. Adrenal, liver, seminal vesicle and prostate weights^{a)} of guinea pigs fed CMA or cortisol in the diet for 33 days (Mean±S. E.)

Sex	Dose (% in the diet)	No.	Final b. w. (g)	Adrenal (mg)	Liver (g)	Seminal vesicle (g)	Prostate (g)
Male	0	7	677.0±20.4	322.3±19.0 (47.6± 2.4)	30.8±1.7 (4.6±0.2)	4.15±0.48 (0.61±0.08)	1.05±0.09 (0.16±0.01)
	CMA	0.01	7	668.3±28.0	355.0±20.6 (53.4± 3.0)	32.7±2.1 (4.9±0.2)	3.58±0.11 (0.54±0.03)
		0.1	8	613.6±28.3	362.3±17.5 (60.1± 4.6)*	30.4±2.0 (4.9±0.1)	1.87±0.28** (0.31±0.04)**
		1.0	5	526.2±23.5**	333.4±19.4 (63.3± 2.4)**	26.4±3.2 (5.0±0.5)	0.79±0.12** (0.15±0.02)**
	Cortisol	0.01	8	586.0±10.0**	242.4± 8.3** (41.5± 1.8)	37.0±1.6** (6.3±0.2)**	4.12±0.44 (0.70±0.07)
		0.1	8	546.1±23.1**	224.6± 8.6** (41.7± 2.3)	47.2±4.1** (8.6±0.5)**	3.40±0.31 (0.63±0.06)
							0.82±0.10 (0.15±0.02)
	Female	0	8	506.9±15.0	248.4±11.3 (49.2± 2.2)	25.6±1.1 (5.1±0.1)	
		CMA	0.01	8	537.4±21.7	247.3±16.9 (45.9± 2.1)	25.5±1.5 (4.7±0.2)
			0.1	8	525.3±23.7	273.3±16.7 (52.3± 3.2)	29.5±1.0* (5.7±0.2)*
			1.0	6	308.2±29.5**	306.5±31.7 (106.8±16.9)*	16.6±2.2** (5.3±0.3)
	Cortisol	0.01	8	462.3±10.9*	229.4±18.9 (49.3± 4.3)	28.9±2.8 (6.2±0.5)	
		0.1	6	477.2±24.9	208.2±24.5 (43.8± 4.7)	49.7±5.5** (10.3±0.7)**	

a) Relative weights to 100 g body weight were shown in parentheses

*: Significantly different from the control (P<0.05)

**: Significantly different from the control (P<0.01)

Table 2. Serum levels of cortisol and total cholesterol in guinea pigs fed CMA or cortisol in the diet for 33 days (Mean±S. E.)

Sex	Dose (% in the diet)	No.	Cortisol (μg/dl)	Total cholesterol (mg/dl)
Male	0	7	141.4±11.6	51.3± 2.5
	CMA	0.01	7	164.2±14.0
		0.1	8	170.0±25.7
		1.0	5	295.3±31.7**
	Cortisol	0.01	8	102.9±11.1**
		0.1	8	72.3± 9.1
Female	0	8	109.5±33.9	64.6± 6.3
	CMA	0.01	8	211.1±24.6
		0.1	8	227.3±30.2
		1.0	3	265.5±29.1
	Cortisol	0.01	7	82.4± 4.6*
		0.1	6	102.3±12.0*

*: Significantly different from the control (P<0.05)

**: Significantly different them the control (P<0.01)

Table 3. Hematological findings of guinea pigs fed CMA or cortisol in the diet for 33 days (Mean±S. E.)

Sex	Dose (% in the diet)	No.	Erythrocyte ($\times 10^4/\text{mm}^3$)	Leucocyte ($\times 10^2/\text{mm}^3$)	Neutrophils (%)	Lymphocytes (%)			
Male	CMA	0	7	421.6 $\pm$ 7.3	85.7 $\pm$ 8.1	20.6 $\pm$ 5.0	76.9 $\pm$ 5.2		
		0.01	7	437.1 $\pm$ 16.3	114.2 $\pm$ 11.7	24.3 $\pm$ 4.2	73.1 $\pm$ 3.9		
		0.1	8	431.9 $\pm$ 12.4	95.3 $\pm$ 9.8	22.0 $\pm$ 4.3	76.1 $\pm$ 4.6		
		1.0	5	461.2 $\pm$ 55.6	95.8 $\pm$ 15.1	30.4 $\pm$ 7.1	68.0 $\pm$ 6.5		
	Cortisol	0.01	8	478.8 $\pm$ 21.1*	84.7 $\pm$ 5.1	39.4 $\pm$ 6.9	59.3 $\pm$ 6.7		
		0.1	8	486.3 $\pm$ 18.6*	87.6 $\pm$ 11.1	58.0 $\pm$ 4.8**	42.0 $\pm$ 4.8**		
		Female	CMA	0	8	384.8 $\pm$ 18.8	93.4 $\pm$ 8.9	15.5 $\pm$ 3.2	79.3 $\pm$ 5.1
				0.01	8	414.4 $\pm$ 19.6	95.3 $\pm$ 7.5	12.9 $\pm$ 3.7	84.6 $\pm$ 4.1
0.1	8			357.3 $\pm$ 17.7	90.2 $\pm$ 10.8	16.0 $\pm$ 3.9	83.1 $\pm$ 3.7		
1.0	3			ND	ND	27.0 $\pm$ 3.6	68.0 $\pm$ 4.7		
Cortisol	0.01		8	462.0 $\pm$ 11.0**	105.2 $\pm$ 11.3	23.6 $\pm$ 4.8	74.4 $\pm$ 4.7		
	0.1		6	494.8 $\pm$ 21.5**	75.9 $\pm$ 2.9	66.3 $\pm$ 4.9**	32.3 $\pm$ 4.2**		

ND: No data

*: Significantly different from the control ($P<0.05$)**: Significantly different from the control ($P<0.01$)

slight thickening of zona fasciculata in most animals at the dose level of 1.0% in each sex, including deceased animals. However, no degenerative signs were noted in its cells (Fig. 1). The thymus (Fig. 1) and liver revealed no abnormality in CMA treated groups. The seminal vesicle and prostate showed a dose-related atrophy of glandular epithelium with the diminished secretion at the dose levels of 0.1 and 1.0% of CMA in males. Endometrial proliferation in uterus was also demonstrated in females treated with CMA. In cortisol treated groups, the adrenal showed marked atrophy of zona fasciculata, indicating shrunken and pyknotic nuclei of its cells in all groups (Fig. 1), whereas zona reticularis was slightly narrowed in some of the males at the dose level of 0.1% of cortisol. The zona glomerulosa showed its normal aspect. The thymic cortex was depressed with a marked decrease in numbers of the lymphocytes in all groups fed cortisol (Fig. 1). The liver showed a dose-related swelling of the hepatocytes throughout the lobules with a foamy appearance of the cytoplasm in each sex treated with cortisol. The seminal vesicle, prostate and uterus revealed no abnormalities in cortisol treated animals.

The large dose of CMA had cortisone-like properties in rats (Usui et al., 1977; Makino et al., 1977), dogs (Usui et al., 1977) and rabbits (Suzuki et al., 1977), such as adrenal and thymic involutions (Selye, 1937), hyperlipemia (Adlersberg et al., 1950) and enlargement of the liver (Falconi et al., 1972), although its mode of anti-inflammatory action (Izumi et al., 1977) and toxicologic signs (Usui et al., 1977) were qualitatively different from those of glucocorticoids. In the present results, a slight acceleration of the adrenocortical function was noted in guinea pigs treated with high dosage of CMA, suggestive non-specific stress of medication. But no signs of adrenocortical insufficiency and cortisone-like effects were noted in all groups fed CMA contained diet. By the treatment with cortisol, however, the atrophy of adrenal and thymic cortex, hypercholes-

Fig. 1. Outer part of the adrenal cortex (a-c) and thymus (d-f) from male guinea pigs. H. E. stain
a-c: $\times 266$, d-f: $\times 102$

- a: Zona glomerulosa and zona fasciculata from a control animal.
- b: Zona glomerulosa and zona fasciculata from an animal fed 1.0% CMA contained diet for 33 days. No signs of the atrophy of zona fasciculata are demonstrated.
- c: Zona glomerulosa, zona fasciculata and outer part of zona reticularis from an animal fed 0.1% cortisol contained diet for 33 days. Marked atrophy of zona fasciculata is noted with degenerative signs of nuclei, such as shrinkage and pyknosis.
- d: Thymic cortex and medulla from a control animal.
- e: Thymic cortex and medulla from an animal fed 1.0% CMA contained diet for 33 days. Normal appearance of the cortex is noted.
- f: Thymic cortex and medulla from an animal fed 0.1% cortisol contained diet for 33 days. Severe atrophy of the cortex with the loss of lymphocytes is noted.

terolemia and the enlargement of the liver were noted in guinea pigs. It was therefore concluded that CMA had progestational and anti-androgenic properties as indicated endometrial proliferation in females and atrophy of sexual accessory glands in males, but not adrenocortical hormone-like ones in guinea pigs. So, it seems likely that the adrenal suppressive action of CMA is a species-specific one.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. Ryuta Suzuki, Medical Manager, for his helpful advice and also to Dr. Yoshiro Hiramatsu, Director of Pharmacological Research Department, Teikoku Hormone Mfg. Co., Ltd., for his encouragement and criticism. The authors are indebted to Mr. Tetsuo Usui, Mr. Mamoru Mieda, Mr. Katsuya Eguchi, Mr. Toshiyuki Yamamoto, Mr. Susumu Ito, Miss Yukiko Yamashita, Miss Mieko Sakka, Miss Noriko Inoue, Miss Hiroko Matsumura and Miss Keiko Tsuji, the members of Pharmacological Research Department, for their laborious cooperations during this work.

REFERENCES

- Adlersberg D., Schaefer L. E. and Ditch R. (1950). Adrenal cortex and lipid metabolism: effects of cortisone and adrenocorticotrophin (ACTH) on serum lipids in man. *Proc. Soc. Exper. Biol. Med.*, 74, 877-879.
- Brennan D. M. and Kraay R. J. (1963). Chlormadinone acetate, a new highly active gestation-supporting agent. *Acta endocr. (Kbn.)*, 44, 367-379.
- Dörner G., Götz F. and Mainz K. (1972). Influence of antiandrogens on sexual behaviour and gonadal function in male rats. *Endocrinol. Exp. (Bratisl.)*, 6, 17-27.
- Dominguez H., Simowitz F. and Greenblatt R. B. (1962). Clinical evaluation of a new oral progestin-chlormadinone. *Am. J. Obstet. Gynec.*, 84, 1478-1486.
- Falconi G., Galletti F. and Celasco G. (1972). Comparative percutaneous toxicity in rabbits of betamethasone 17-benzoate and other topical anti-inflammatory corticosteroids. In: *Proceedings of the European Society for the Study of Drug Toxicity, Vol. XIII*, pp 301-306. Excerpta Medica Amsterdam.
- Geller J., Fruchtman B., Meyer C. and Newman H. (1967). Effect of progestational agents on gonadal and adrenal cortical function in patients with benign prostatic hypertrophy and carcinoma of the prostate. *J. Clin. Endocr.*, 27, 556-560.
- Givner M. L. and Rochefort L. G. (1972). Effect of synthetic progestogens and estradiol-17 β on adrenal function in the rat. *Endocrinology*, 90, 1238-1244.
- Glenn E. M., Richardson S. L. and Bowman B. J. (1959). Biologic activity of 6-alpha-methyl compounds corresponding to progesterone, 17-alpha-hydroxyprogesterone acetate and compound S. *Metabolism*, 42, 912-917.
- Izumi A., Miyasaka K., Mikami T., Himori N., Ishimori T., Iizuka K. and Hiramatsu Y. (1977). General pharmacological studies on chlormadinone acetate. *Laboratory and Clinic*, 11, 556-570.
- Makino M., Usui T., Horiuchi T., Nagai M., Eguchi K., Kusunoki F. and Suzuki M. R. (1977). Toxicological studies of chlormadinone acetate. 2. Chronic toxicity study in rats. *Laboratory and Clinic*, 11, 588-608.
- Reissert P.-M. und Mühlen A. v. z. (1969). Untersuchungen über die Gonaden- und Nebennierenrindenfunktion unter einer Gestagen-Therapie (Chlormadinonacetat). *Arzneim.-Forsch.*, 19, 1279-1283.

Chlormadinone and cortisol

- Selye H. (1937). Studies on adaptation. *Endocrinology*, 21, 169-188.
- Suzuki M.R., Usui T., Eguchi K., Yamamoto T., Makino M., Inoue N., Sakka M., Matsumura H. and Horiuchi T. (1977). Toxicity studies of chlormadinone acetate. 5. Teratological study in rats and rabbits. *Pharmacometrics*, in press.
- Usui T., Makino M., Horiuchi T., Eguchi K., Nagai M., Kusunoki F., Kambegawa A., Suzuki M.R., Watanabe K., Komatsu N. and Hasegawa H. (1977). Toxicological studies of chlormadinone acetate. 3. Chronic toxicity study in beagle dogs. *Pharmacometrics*, in press.
- Usui T., Makino M., Horiuchi T., Nagai M., Eguchi K., Kusunoki F., Manita H. and Suzuki M.R. (1977). Toxicological studies of chlormadinone acetate. 1. Acute toxicity, antigenicity and sub-acute toxicity studies. *Laboratory and Clinic*, 11, 571-587.