

Low dose of cyproterone acetate and testosterone enanthate for contraception in men

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After a control phase, 10 normal men received cyproterone acetate (CPA) at a dose of 25 mg/day (CPA-25; $n = 5$) or 12.5 mg/day (CPA-12.5; $n = 5$) plus testosterone enanthate (TE) 100 mg/week, for 16 weeks. Throughout the study sperm counts were performed every 2 weeks, and luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, biochemical and haematological tests were performed every 4 weeks. All five men in group CPA-25 and three men in group CPA-12.5 achieved azoospermia. One man in group CPA-25 was azoospermic by week 12 of hormone administration, but had a sperm count of $0.1 \times 10^6/\text{ml}$ at week 16. Time to azoospermia was 9.0 ± 1.3 and 8.7 ± 0.7 weeks in groups CPA-25 and CPA-12.5 respectively. Gonadotrophins were decreased by week 4 of hormone administration, remained around the minimum detectability of the assay for the duration of hormone administration and returned to baseline after stopping hormone administration. Testosterone values did not change. No change in any biochemical parameters was found. Haematological parameters were decreased at week 16 of hormone administration and returned to baseline after stopping hormone administration. In conclusion, these results suggest that an hormonal regimen consisting of testosterone plus a progestin with anti-androgenic properties holds promise as an effective, safe and reversible male contraceptive.

Key words: contraception/cyproterone acetate/gonadotrophins/spermatogenesis/testosterone

Introduction

Recent studies have shown that the administration of a progestin in combination with an androgen is more effective than that of an androgen alone in suppressing spermatogenesis (Bebb *et al.*, 1996; Meriggiola *et al.*, 1996; Meriggiola and Bremner, 1997). The rationale for this hormonal combination is based on the additive effect of these two compounds in suppressing

gonadotrophins and therefore spermatogenesis. Because of this combined effect, the addition of a progestin allows the use of lower and more physiological doses of testosterone without reducing the suppression of gonadotrophins. This would minimize the incidence of androgen-related side-effects. Research in this field is aimed at both defining the minimum testosterone dose and at selecting the optimal progestin to be used in contraceptive regimens for men.

Among all compounds tested so far, the progestin that has provided the best results both in terms of spermatogenic suppression and in terms of absence of adverse effects is cyproterone acetate (CPA) (Roy *et al.*, 1976; Roy, 1985; Meriggiola *et al.*, 1996). In combination with testosterone enanthate (TE), CPA at high doses induces a more profound, rapid and consistent suppression of spermatogenesis than other regimens. No adverse effects on metabolic parameters were reported except a slight decrease in body weight and in haematological parameters, which seemed to be dependent on the dose of CPA. These effects could be due in part to the fact that CPA is also an anti-androgen.

In the work reported here, we studied the effects of lower doses of CPA than those administered previously in combination with the same dose of TE (100 mg/week), on spermatogenesis, gonadotrophins and metabolic and haematological parameters.

Materials and methods

Subjects

Ten normal Caucasian men, aged 19–42 years (31.4 ± 2.1 ; mean \pm SE) were enrolled in this study. All men were healthy by medical history, physical examination and screening laboratory tests. All of the men had basal sperm counts of $>20 \times 10^6/\text{ml}$ as well as gonadotrophins and testosterone concentrations within the normal range. The study was approved by the Ethical Committee of the S.Orsola Hospital in Bologna, and each man signed an informed consent form.

Clinical protocol

The study protocol consisted of a control period, a 16 week treatment period and a recovery period that lasted until subjects had at least two sperm counts within their own baseline range. During the control phase, subjects provided three seminal fluid samples separated from each other by ≥ 7 days. Three fasting blood samples separated by at ≥ 1 week were obtained. During the treatment phase, the subjects provided seminal fluids every 2 weeks and fasting (≥ 10 h) blood samples every 4 weeks. Blood samples were obtained immediately before the weekly injections of TE were administered. Samples were stored at -20°C until assayed. Every 4 weeks, volunteers attended the clinic to undergo physical examination, weight and blood pressure

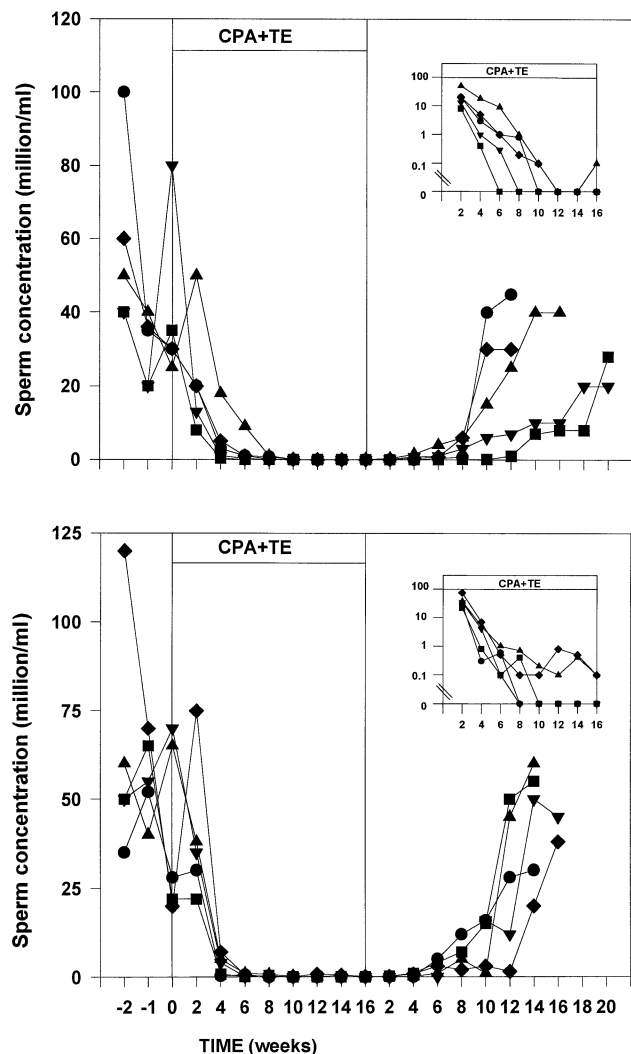


Figure 1. Mean \pm SE sperm concentration at baseline, throughout treatment period and during the recovery phase in the two groups of men: cyproterone acetate (CPA)-25, ●; CPA-12.5, ■.

recording. Volunteers were also asked to complete a sexual and behavioural questionnaire each month (Bagatell *et al.*, 1994).

After the control period, subjects were randomly assigned to receive: (i) CPA 25 mg/ day orally, plus TE 100 mg/week i.m. (CPA-25); or (ii) CPA 12.5 mg/ day orally, plus TE 100 mg/week (CPA-12.5). TE (Test-enant; Geymonat, Frosinone, Italy) was administered in a sesame oil suspension of 1 ml i.m. weekly. CPA (Androcur, Schering, Italy) was taken orally.

Measurements

Semen samples were analysed according to World Health Organization (WHO, 1992) guidelines. Azoospermia was defined as no spermatozoa found in a sample after centrifugation and analysis of the pellet. Recovery of sperm count was calculated considering the first of at least two sperm counts within the baseline range of each subject. Recovery was considered complete when each subject had at least two sperm counts within his own baseline range. Estimation of testis size was performed by orchimeter. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) and testosterone were measured according to previously described methodologies (Meriggiola *et al.*, 1996). The sensitivity of the LH assay was 0.1 IU/l. The sensitivity of the FSH assay was 0.3 IU/l. Haematology (haemoglobin, haemato-

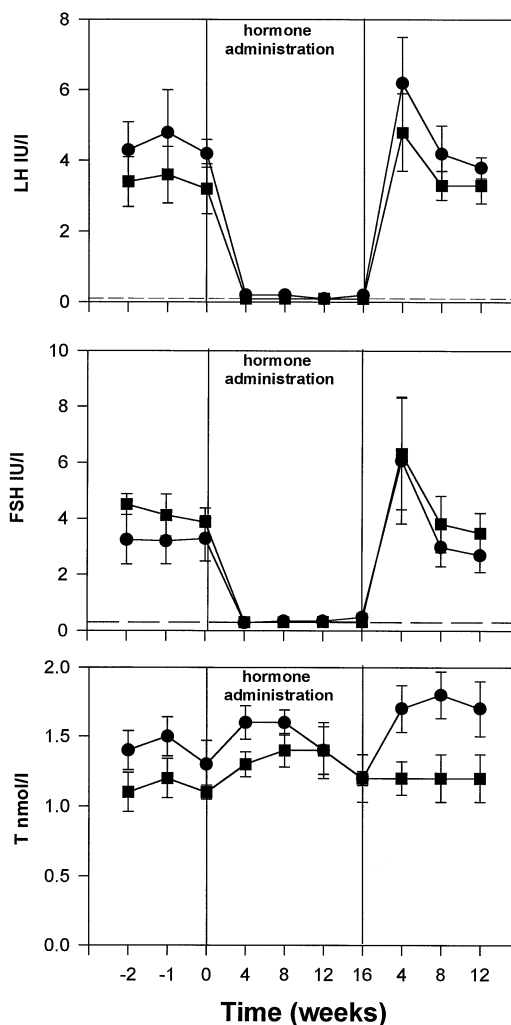


Figure 2. Mean \pm SE values of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels throughout the study periods in the cyproterone acetate (CPA)-25 (●) and CPA-12.5 (■) groups.

crit, red blood cell), chemistry (total cholesterol, high density lipoprotein, triglycerides, urea, creatinine, glutamic oxaloacetate transaminase, glutamic pyruvic transaminase, alkaline phosphatase, bilirubin) and electrolytes (Na, K, Ca, Phosphate) were also measured according to previously validated methodologies (Burlina, 1990).

Statistics

Multifactorial analysis of variance with repeated measures or *t*-test was used to determine differences within each treatment group across time and between study groups of any parameter. Where appropriate, data were log-transformed before analysis.

Results

Semen parameters

Baseline sperm concentration was $42.7 \pm 10.7 \times 10^6$ /ml in group CPA-25 and $53.5 \pm 6.4 \times 10^6$ /ml (mean \pm SE) in group CPA-12.5 [no significant difference (n.s.)] (Figure 1). All five men in group CPA-25 and three out of five men in group CPA-12.5 achieved azoospermia by week 16. One man (CPA 18) of group CPA-25 had no spermatozoa in the ejaculate at

Table I. Laboratory tests throughout the study period in the two groups of men

	CPA 25 mg/day + TE 100 mg/week			CPA 12.5 mg/day + TE 100 mg/week		
	Baseline	Week 16	Recovery	Baseline	Week 16	Recovery
Urea (mmol/l)	6.7±0.0	6.7±0.0	6.7±0.0	5.0±0.0	5.0±0.0	6.7±0.0
Creatinine (µmol/l)	88.4 ± 0.0	88.4 ± 0.0	97.2 ± 0.0	97.3 ± 0.0	88.4 ± 0.0	106.1 ± 0.0
Cholesterol (nmol/l)	4.8 ± 0.2	4.5 ± 0.5	5.1 ± 0.8	5.4 ± 0.3	4.7 ± 0.3	5.3 ± 0.3
Triglycerides (nmol/l)	2.3 ± 0.3	2.0 ± 0.3	2.4 ± 0.3	2.7 ± 0.2	1.9 ± 0.2*	2.8 ± 0.4
HDL (nmol/l)	1.4 ± 0.0	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
LDL (nmol/l)	2.8 ± 0.1	2.8 ± 0.5	3.3 ± 0.7	3.7 ± 0.3	3.1 ± 0.3	3.4 ± 0.3
Total bilirubin (µmol/l)	16.6 ± 0.9	15.1 ± 2.1	15.1 ± 1.7	18.8 ± 3.4	20.5 ± 5.1	17.1 ± 3.4
GOT (U/l)	18 ± 0.5	17.0 ± 1.7	20.2 ± 1.0	23.0 ± 2.0	18.0 ± 1.0	23.0 ± 3.0
GPT (U/l)	15.0 ± 1.0	16.0 ± 4.0	22.0 ± 3.0	19.0 ± 2.0	18.0 ± 3.0	22.0 ± 3.0
Calcium (mmol/l)	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.1	2.5 ± 0.0	2.4 ± 0.0	2.5 ± 0.0
Phosphate (mmol/l)	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.1
Potassium (mmol/l)	4.1 ± 0.1	4.2 ± 0.1	4.6 ± 0.2	4.3 ± 0.0	4.2 ± 0.1	4.8 ± 0.4
Sodium (mmol/l)	142 ± 0	136 ± 4	144 ± 1	140 ± 1	141 ± 1	144 ± 1
Haemoglobin (g/l)	148 ± 1	142 ± 2*	146 ± 7	152 ± 3	148 ± 3	155 ± 3
Haematocrit (%)	43 ± 1	41.1*	43 ± 2	45 ± 1	43 ± 1	45 ± 1
Red blood cells (×10 ⁶ µl)	5.0 ± 0.1	4.8 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	4.9 ± 0.2	5.1 ± 0.2

*Significantly different ($P < 0.05$) from baseline.

CPA = cyproterone acetate; TE = testosterone enanthate; HDL = high density lipoprotein; LDL = low density lipoprotein; GOT = glutamic oxaloacetate transaminase; GPT = glutamic pyruvic transaminase.

weeks 12 and 14 but had 0.1×10^6 /ml at week 16. Two men of the CPA-12.5 group never achieved azoospermia and both had sperm counts of 0.1×10^6 /ml at week 16. Mean time to achieve azoospermia was 9.0 ± 1.3 and 8.7 ± 0.7 weeks in groups CPA-25 and CPA-12.5 respectively. After stopping hormone administration, sperm counts returned to baseline levels in all men. Mean time to return to baseline was 14.8 ± 2.2 and 12.8 ± 0.5 weeks in the CPA-25 and CPA 12.5 groups respectively (n.s.).

Hormone concentrations

LH values at baseline were 4.4 ± 0.5 and 3.4 ± 0.4 IU/l (mean ± SE) in the CPA-25 and CPA-12.5 groups respectively (n.s.). FSH values at baseline were 3.2 ± 0.4 and 4.2 ± 0.3 IU/l in the CPA-25 and CPA-12.5 groups respectively (n.s.). Testosterone values at baseline were 16.7 ± 1.04 and 13.5 ± 0.69 nmol/l in the CPA-25 and CPA-12.5 groups respectively (n.s.). After 4 weeks of hormone administration, LH and FSH serum values were already significantly decreased and stayed around the minimum detectability of the assay throughout the entire duration of the treatment (Figure 2). At week 16, one man (CPA18) had higher FSH values (1.0 IU/l) compared to other subjects and to his own FSH values during the other weeks of the study. No significant difference in gonadotrophin serum concentrations was found between the two groups in any phase of the study. Levels of both gonadotrophins returned to baseline by week 4 of the recovery phase. Testosterone serum levels did not show any significant change in any phase of the study in either group.

Lipids, blood chemistry, electrolytes and haematology

No change in metabolic parameters, including lipids, was detected in any group (Table I). No change in serum electrolyte values could be found. Haemoglobin and haematocrit were slightly decreased at week 16 of hormone administration compared to baseline in both groups (CPA-25, $P < 0.05$; CPA-

12.5, n.s.). Haemoglobin decreased by 4.1 and 2.6%, and haematocrit decreased by 5.5 and 3.8% in the CPA-25 and CPA-12.5 groups respectively. These changes returned to baseline by week 12 of recovery.

Clinical parameters

Testicular size decreased in all subjects during hormone administration. Mean baseline testis volumes were 20.0 ± 0.5 ml and 22.0 ± 0.8 ml in groups CPA-25 and CPA-12.5 respectively (mean ± SE of left and right testis). At week 16 of hormone administration testis volumes were 12.0 ± 1.0 and 15.0 ± 0.0 ml in the two groups respectively (percentage decreases were 34 ± 6 and 31 ± 4). Testis size returned to normal in all subjects after stopping hormone administration. No change of body weight was registered in any subject of any group. No change in any parameters of sexual function and behaviour was reported in any subject of any group.

Discussion

Our results show that CPA, administered at the doses of 25 and 12.5 mg/day in combination with TE 100 mg/week, induces a profound suppression of sperm production in all men. No major adverse effects were detected during the 16 weeks of hormone administration with either dose of CPA. A slight decrease in haematological parameters was still present with the lowest dose of CPA that we used. This study confirms and extends previously reported results and suggests that this hormonal combination represents a highly promising contraceptive regimen for men.

In a previous study we demonstrated that CPA, administered at higher doses (100 or 50 mg/day) in combination with TE 100 mg/week, induces a profound, rapid and consistent suppression of spermatogenesis in all tested men (Meriggiola *et al.*, 1996). The results of that study showed that this hormonal regimen is more effective not only compared to

androgen-alone regimens but also compared to combined regimens that used other progestins such as medroxyprogesterone acetate (MPA) (Wu and Aitken, 1989) or levonorgestrel (LNG) (Bebb *et al.*, 1995). Although the mechanism by which this hormonal combination acts is not certain, we postulate that both the progestational and the anti-androgenic effect of CPA are important for the achievement of the profound suppression of spermatogenesis. Because of its progestational activity CPA may act together with TE at the hypothalamus–pituitary level in suppressing gonadotrophins (Matsumoto and Bremner, 1989). Because of its anti-androgenic activity CPA may also act at the gonadal level to block the stimulatory effect of androgens on sperm development (Whalen and Lutge, 1969; Sharpe, 1987; Sharpe *et al.*, 1988; Weinbauer *et al.*, 1988; Anderson *et al.*, 1996, 1997; Meriggiola and Bremner, 1997). Compared to previous studies that used MPA or LNG, the administration of CPA with testosterone resulted not only in a better suppression of spermatogenesis but also in fewer adverse effects (Friedl *et al.*, 1985; Bebb *et al.*, 1996). We did not observe any effects on lipids, weight gain or acne equivalent to those which were reported when 19-nor-derived progestins were used. The absence of androgen-related adverse effects might be due to either the more physiological dosage of TE (100 mg/week) that we used in this study (Sokol *et al.*, 1982; Matsumoto, 1990) or to the anti-androgenic effects of CPA. The only adverse effect that we recorded when 100 or 50 mg/day of CPA were administered with TE 100 mg/week, was a slight decrease in body weight and in haematological parameters that seemed to be dependent on the dose of CPA.

Therefore, in this study we tested the hypothesis that decreasing the dose of CPA would still induce a profound suppression of spermatogenesis and at the same time avoid adverse effects such as those on body weight and haematological parameters. Both the CPA doses that we tested in this study provided a very profound suppression of gonadotrophins and of spermatogenesis. At the dose of 25 mg/day, CPA induced azoospermia in all men by week 12 of hormone administration. In one man sperm count returned to 0.1×10^6 /ml on the last day of hormone administration in week 16. On the same day, gonadotrophin levels were slightly higher compared to those measured in weeks 4, 8 and 12. The volunteer did not report forgetting any pill throughout the treatment period and he regularly underwent injections. The reason for the escape of spermatogenesis from suppression in this subject is not clear. The spermatogenetic process requires ~72 days in humans. Therefore, it seems unlikely that a gonadotrophin increase during week 16 would have resulted in the maturation of some sperms. In this subject, gonadotrophin values were suppressed in weeks 4, 8 and 12, as in all the other subjects. However, sperm cells blocked by the anti-androgen CPA at an advanced stage of maturation might require less time and less gonadotrophins to complete their maturation.

Roy (1985), who used 20 mg/day of CPA in combination with TE 250 mg/2 weeks, showed that five out of six subjects became azoospermic following 8 weeks of hormone administration, while in one subject sperm count was 0.01×10^6 /ml. Our work and Roy's data taken together suggest that this

hormonal regimen promises to be more effective than all previously tested regimens, based either on the administration of testosterone alone or testosterone plus a progestin. In a recently published study it was shown that there is a close relationship between sperm count and pregnancy rate following administration of TE 200 mg/week (WHO, 1990, 1996). In that study sperm count of $<3 \times 10^6$ /ml and azoospermia provided a Pearl rate of 1.4 (C.I. 0.4–3.7) per 100 persons/year. If we apply these results to our study, it appears that even at the lowest dose used in this study, this regimen is likely to have a very high contraceptive effectiveness, perhaps comparable to that of oral female contraceptives.

It is of some interest to notice that, regardless of the dose that we used, this hormonal regimen was not only more effective but also caused fewer and less important adverse effects than the administration of TE alone both at the dose of 100 mg/week (Bebb *et al.*, 1996; Meriggiola *et al.*, 1996) and of 200 mg/week (Bagatell *et al.*, 1994; Meriggiola *et al.*, 1995). In this study no major adverse effects were reported with any dose. We found a 4.1 and 2.6% decrease in haemoglobin and 5.5 and 3.8% decrease in haematocrit in the group CPA-25 and CPA-12.5 respectively. The data on haematological parameters confirm those of our previous study and suggest that the decrease in haemoglobin and haematocrit is dependent on the dose of CPA.

In conclusion, the results of this study confirm our previously reported work showing that a hormonal regimen consisting of testosterone administered together with a progestin with anti-androgenic properties very effectively suppresses spermatogenesis without causing major adverse effects.

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