0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(5):2254–2262

doi: 10.1210/jc.2003-031307

Male Hormonal Contraception: Effects of Injections of Testosterone Undecanoate and Depot Medroxyprogesterone Acetate at Eight-Week Intervals in Chinese Men

YI-QUN GU, JIAN-SUN TONG, DING-ZHI MA, XING-HAI WANG, DONG YUAN, WEN-HAO TANG, AND WILLIAM J. BREMNER

National Research Institute for Family Planning (Y.-Q.G., D.Y., W.-H.T.), Beijing 100081, China; Jiangsu Family Planning Research Institute (J.-S.T., D.-Z.M., X.-H.W.), Jiangsu 210036, China; and Department of Medicine (W.J.B.), University of Washington, Seattle, Washington 98195

Surveys indicate that one form of acceptable male hormonal contraception would consist of injections given at 2- to 3-month intervals. This report describes a study of depot medroxyprogesterone acetate (DMPA) and testosterone undecanoate (TU) injected at 8-wk intervals for suppression of spermatogenesis in healthy Chinese men. After screening, 30 healthy volunteers were enrolled and randomly assigned to one of three dose groups (n = 10/group): 1000 mg TU (group A); 1000 mg TU plus 150 mg DMPA (group B); 1000 mg TU plus 300 mg DMPA (group C). All doses were given as im injections at 8-wk intervals. The study consisted of an 8-wk control (baseline) period, a 24-wk treatment period, and a 24-wk recovery period. Consistent azoospermia or severe oligozoospermia was achieved and maintained in all volunteers during the treatment period, except for two men in the TU-alone group who experienced a rebound in sperm concentrations. An 8-wk regimen of TU plus DMPA at both tested combination doses effectively suppressed spermatogenesis to azoospermia in Chinese men. All volunteers tolerated the injections; no serious adverse effects were reported. The lower-dose combination is recommended for further testing in an expanded clinical trial or contraceptive efficacy study. (*J Clin Endocrinol Metab* 89: 2254–2262, 2004)

A NUMBER OF approaches to male hormonal contraception using testosterone (T) esters have been widely investigated (1–7). In several studies, an androgen ester has been administered to both consistently suppress gonadotropins and serve as androgen replacement. Azoospermia could be achieved in 60% of Caucasian men and 91% of Chinese men by administration of weekly T enanthate (TE) im injections in a multicenter clinical study (8). Recently a multicenter clinical contraceptive efficacy study (9) completed in China demonstrated a 5.2% failure rate resulting from the administration of monthly im injections of 500 mg T undecanoate (TU), a stable and long-acting T ester (10). However, most men who participated in this study expressed a preference for a longer-acting regimen, for example with 2- to 3-month injection intervals (9).

Results from several studies suggested a direct relationship between sperm suppression and gonadotropin suppression (11, 12). Profound suppression of spermatogenesis can be achieved by the addition of a progestin to an androgen, to act synergistically on the hypothalamus and pituitary to suppress gonadotropin secretion (13). A trial of levonorgestrel (LNG), given in conjunction with TE, demonstrated that the LNG-TE combination was superior to TE alone in terms of producing azoospermia and severe oligozoospermia as well as the speed at which these end points were produced (11). Equally good results were obtained with lower doses of LNG plus TE (11, 14).

In the 1970s, trials of monthly im injections of depot medroxyprogesterone acetate (DMPA), combined with weekly im injections of TE did not demonstrate a profound suppression of spermatogenesis in Caucasian men at any of several dose combinations (15–17); this finding may be related to the pharmacokinetics of the short-acting TE formulation.

To optimize the suppression of spermatogenesis, 200 mg DMPA was administered monthly in combination with 250 mg of the long-acting formulation of TU in tea seed oil. This combination regimen produced azoospermia in 100% of the 14 Chinese volunteers within 1–4 months (18). An alternative regimen consisting of DMPA given in conjunction with sc T pellets was evaluated; this combination produced a rapid and profound suppression of gonadotropins and induction of azoospermia with no major adverse effects (12). In a more recent dose-finding study in Indonesia, spermatogenesis was rapidly and completely suppressed after the administration of 500 mg TU in tea seed oil every 6 wk in combination with 250 mg DMPA every 12 wk (19).

The availability of long-acting injectable TU preparations in tea seed oil, given at doses of 1000 mg, could allow the development of an 8-wk injection regimen for male contra-

Abbreviations: DMPA, Depot medroxyprogesterone acetate; E_2, estradiol; HDL, high-density lipoprotein; LNG, levonorgestrel; MPA, medroxyprogesterone acetate; PSA, prostate-specific antigen; T, testosterone; TE, testosterone enanthate; TU, testosterone undecanoate; WHO, World Health Organization.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

ception in Chinese men (10). However, this TU formulation would need to be administered in combination with a progestin such as DMPA if it is to be considered an 8-wk regimen because TU alone at dose of 1000 mg failed to profoundly suppress gonadotropins for as long as 8 wk (10).

The promising results of previous studies of DMPA in combination with long-acting androgen preparations encouraged us to perform a clinical trial to assess the contraceptive potential of an 8-wk regimen consisting of a long-acting progestin, DMPA, and a long-acting androgen, injectable TU in tea seed oil, in healthy Chinese men. It was hypothesized that such a combination could lead to improved effectiveness and acceptability of a combination hormonal male contraceptive method by improving the suppression of gonadotropins and reducing the injection frequency and androgen-related adverse effects.

Subjects and Methods

Subjects and allocation

This study was sponsored by the Contraceptive Research and Development Program, as a joint study between a developing country (China) and a reproductive research center in the United States. This study was approved by the Institutional Review Board and Scientific and Ethical Review Group of the University of Washington and the National Research Institute for Family Planning. All volunteers were recruited by and the study was performed at the Jiangsu Family Planning Research Institute, Jiangsu, China.

Of 48 potential volunteers who were screened, 18 were ineligible according to study entry criteria due to abnormality of semen parameters (7), hepatitis (3), abnormality of blood lipids (3), or inability to undergo regular injections and follow-up (5). Thirty healthy Chinese men aged 20–45 yr were enrolled into this study. All 30 men had normal medical histories, physical examination, and laboratory test results. All of them demonstrated normal reproductive functions as evidenced by basal sperm concentrations greater than 20×10^6/ml, sperm motility greater than 50% (grades a+b), and morphology (normal forms) greater than 30% as well as serum gonadotropins and T levels within the normal range for the clinical trial center. Informed consent was obtained from the volunteers and their partners at admission into the trial. According to a randomization table, the 30 men were assigned to one of three dose groups, with 10 volunteers per group, as follows: 1000 mg TU (group A); 1000 mg TU plus 150 mg DMPA (group B); 1000 mg TU plus 300 mg DMPA (group C). All doses were given as im injections at 8-wk intervals.

Androgen/progestin preparation

Injectable TU was provided by Zhejiang Xian Ju Pharmaceutical Corp. (Zhejiang, China). This TU preparation, manufactured under the Chinese Pharmacopeia, was provided in ampoules containing 250 mg of the ester in 2 ml tea seed oil (Mellaleuca). The same batch of TU was used throughout the study.

DMPA was manufactured and distributed by Pharmacia Corp. (Peapack, NJ). The registered trade name for this product is Depo-Provera. The compound was formulated as an aqueous suspension and packaged in 1-ml vials containing 150 mg DMPA.

Study protocol

This was a Phase I, open-label, randomized, dose-finding clinical study consisting of an 8-wk control period, a 24-wk treatment period, and a 24-wk recovery period. During the control period, the volunteers were asked to provide two semen samples by masturbation after 2–7 d of sexual abstinence; samples were provided at 2-wk intervals. Fasting (at least 10 h) blood samples were obtained for baseline measurements of routine hematological profiles, hormone concentrations, medroxyprogesterone acetate (MPA), prostate-specific antigen (PSA), plasma lipids, and blood chemistry. Volunteers underwent a general physical examination and andrological examination, with inspection of external genitalia and measurements of testicular volume, breast size, and tenderness. Transrectal ultrasonography of the prostate was performed in all volunteers at baseline and thereafter at the end of each study period.

The treatment period was defined as commencing with the first hormone administration. During the treatment period, volunteers in the two combination groups received either 150 or 300 mg DMPA injections at 8-wk intervals, and all volunteers received TU injections at a dose of 1000 mg at 8-wk intervals. All volunteers entering this period continued to use their previous contraceptive methods; they attended the clinic every 4 wk to undergo physical and andrological examinations, weight, and blood pressure measurements. During each visit, volunteers provided a semen sample and a fasting blood sample. In addition, volunteers in the two combination groups provided blood samples for serum MPA assays at weekly intervals for 8 successive wk from the beginning of the treatment period. Blood samples were drawn before the subsequent hormone injections were administered.

The recovery period commenced at the end of the treatment period. During the recovery period, after cessation of hormone administration, each volunteer attended the clinic to undergo a physical examination and blood and semen sample collection at 4-wk intervals until the semen parameters of each volunteer returned to his own baseline levels or the normal reference range or until the end of the recovery period.

Measurements

Semen analyses were performed according to the procedures outlined in the World Health Organization (WHO) laboratory manual for the examination of human semen and sperm-cervical mucus interaction (20). Azoospermia was defined as the absence of sperm in seminal fluid, even after centrifugation. Severe oligozoospermia was defined as sperm concentrations of 3×10^6/ml or less. Sperm rebound was defined as the appearance of sperm in the ejaculate at a concentration greater than 3×10^6/ml, after a period of azoospermia or severe oligozoospermia; for verification, repeat examinations were performed 1–2 wk following the time at which the first semen sample in which a rebound was suspected was provided. The volume of each testis was estimated by a Prader's orchidometer, and measurements were combined to give the total testicular volume. The transverse, sagittal, and anteroposterior diameters of the prostate were measured by transrectal ultrasonography, and the results were recorded. The total prostate volume was calculated with the standard formula (21). Serum T, estradiol (E_2), LH, FSH, and PSA were measured by commercial kits supplied by Biodata S.p.A. (Rome, Italy). The assay sensitivities were 0.3 nmol/liter, 18 pmol/liter, 0.2 IU/liter, 0.3 IU/liter, and 0.1 ng/ml for T, E_2, LH, FSH, and PSA, respectively. The mean intraassay coefficients of variation for serum T, E_2, LH, FSH, and PSA were 1.8, 4.4, 4.9, 4.6, and 3.8%, respectively. The mean interassay coefficient of variation was less than 8.6% for the assays of all five hormones. Serum SHBG concentrations were measured by commercial kits supplied by Euro-Diagnostica (Malmö, Sweden). The mean intraassay and interassay coefficients of variation for serum SHBG were 4.1 and 8.3%, respectively. Free T concentrations were calculated based on a formula (22). Serum MPA concentrations were measured by commercial kits supplied by Immunometrics Ltd. (London, UK). The assay sensitivity was 150 pmol/liter. The mean intraassay and interassay coefficients of variation for serum MPA assays were 4.7 and 4.3%, respectively. Total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein cholesterol were measured by the method of phosphotungstic acid magnesium precipitation; the normal ranges are 3.10–5.69, 0.83–1.96, and 1.90–3.80 mmol/liter, respectively.

Statistical analysis

Data processing included double input of the data in the programmed data bank, coding of accompanying illnesses, and any reasons for discontinuation. Multifactorial and one-way ANOVA, followed by *post hoc* test (V8 software package, SPSS, Inc., Chicago, IL) were used to determine differences across time and groups for any parameter. Sperm concentration and LH and FSH data were log transformed before analysis. Results are expressed as the mean ± SEM. $P < 0.05$ was considered to be significant.

Results

Clinical features

All 30 volunteers tolerated the injections; there were no early withdrawals or losses to follow-up.

No serious adverse event occurred during the study. The most commonly reported side effect was tenderness or discomfort at the injection sites. This usually occurred after one or two injections and then disappeared gradually. No change in sexual desire was reported, and no men complained of changes in mood or the development of aggressive behavior.

Gynecomastia was diagnosed in two volunteers (one in group B and one in group C) in the early stage of the recovery period. A blood sample for measurement of serum E_2 was obtained, and antiestrogen treatment was given to these two volunteers for 2–3 wk. Both cases completely resolved by the end of the recovery period.

The changes in mean body weight and total testicular volume over the course of the study are shown in Table 1. Mean body weight slightly increased in all groups, with a maximum increment of 1.4 kg during the treatment period and a gradual return toward the baseline values after cessation of the injections. Mean total testicular volume slightly decreased in all groups, with a maximum decrement of approximately 1 ml during the treatment period followed by a return toward the baseline levels during the recovery period. Prostate volumes remained unchanged throughout the study in all groups (Table 1) and were similar to the normal reference value (14.6 ± 0.2 ml) in healthy Chinese men aged between 20 and 45 yr (21).

Sperm concentrations

Mean sperm concentrations in each group were gradually reduced after the injections of the first regimen, and a significant decrease was found at the 12th wk of the treatment period when compared with baseline values. Mean sperm concentrations were maintained at very low levels throughout the remaining 12 wk of the treatment period (Fig. 1). The mean waiting time for the onset of spermatogenic suppression was 92 ± 6, 80 ± 5, and 83 ± 8 d for groups A, B, and C, respectively; these values were not statistically different. Distribution of the volunteers and time for achieving azoospermia or severe oligozoospermia during the treatment period are shown in Table 2. Azoospermia or severe oligozoospermia was achieved and maintained in all volunteers during the treatment period, but sperm rebound occurred in two men (sperm concentrations were 11×10^6/ml and 19×10^6/ml, respectively) in the TU-alone group. There was a trend toward a more sustained suppression of spermatogenesis in the TU/DMPA combination groups than in the TU-alone group, although the difference was not significant. Spermatogenesis started to recover during the recovery period. However, recovery of spermatogenesis in the two combination groups was delayed, compared with that in the TU-alone group, and azoospermia or severe oligozoospermia was maintained for an additional 8 wk after cessation of the treatment period (Fig. 1). By the 16th wk of the recovery period, mean sperm concentrations in the three groups had returned to the normal reference range (20×10^6/ml), and there was no significant difference in this parameter when

TABLE 1. Parameters in each group in control, treatment, and recovery period

	1000 mg TU			1000 mg TU + 150 mg DMPA			1000 mg TU + 300 mg DMPA		
	Control	Treatment	Recovery	Control	Treatment	Recovery	Control	Treatment	Recovery
CHO (mmol/liter)	4.35 ± 0.22	4.44 ± 0.10	5.01 ± 0.12	4.59 ± 0.27	4.38 ± 0.15	5.20 ± 0.16	4.08 ± 0.43	4.35 ± 0.15	5.04 ± 0.15[a]
HDL (mmol/liter)	2.15 ± 0.16	1.76 ± 0.06[a]	1.57 ± 0.05[a]	2.21 ± 0.20	1.78 ± 0.07[a]	1.44 ± 0.05[a]	2.01 ± 0.17	1.66 ± 0.06[a]	1.45 ± 0.05
LDL (mmol/liter)	2.73 ± 0.40	2.27 ± 0.11	2.92 ± 0.12	3.17 ± 0.57	2.26 ± 0.09[a]	3.08 ± 0.10	2.43 ± 0.26	1.99 ± 0.07	2.96 ± 0.09
TRIG (mmol/liter)	1.79 ± 0.42	1.50 ± 0.10	1.79 ± 0.13	2.13 ± 0.52	1.49 ± 0.08	1.77 ± 0.10	1.38 ± 0.22	1.23 ± 0.07	1.72 ± 0.08
ALT (U/liter)	26.40 ± 5.95	20.63 ± 0.97	23.35 ± 1.28	31.50 ± 5.45	21.48 ± 1.47[a]	29.63 ± 1.50	24.60 ± 4.06	22.15 ± 1.35	27.80 ± 1.73[a]
BUN (mmol/liter)	4.67 ± 0.24	4.77 ± 0.17	5.65 ± 0.23[a]	5.35 ± 0.38	4.51 ± 0.20	5.89 ± 0.21[a]	4.51 ± 0.29	4.41 ± 0.17	5.26 ± 0.21[a]
Hb (g/dl)	12.75 ± 0.29	14.27 ± 0.14[a]	13.37 ± 0.11	12.10 ± 0.40	12.67 ± 0.17	12.37 ± 0.13	13.40 ± 0.33	13.55 ± 0.12	13.35 ± 0.12
Hematocrit (%)	41.50 ± 1.36	44.08 ± 0.57	42.13 ± 0.50	38.75 ± 0.70	40.13 ± 0.50	39.35 ± 0.27	41.30 ± 1.25	43.00 ± 0.63	42.48 ± 0.57
BW (kg)	67.10 ± 2.54	68.38 ± 0.94	67.47 ± 0.95	71.70 ± 1.71	72.18 ± 0.65	71.70 ± 0.59	70.90 ± 2.47	72.27 ± 0.83	71.50 ± 0.85
Testis volume (ml)	35.50 ± 1.57	35.17 ± 0.69	35.67 ± 0.80	40.50 ± 1.57	39.25 ± 0.44	39.00 ± 0.39	42.00 ± 2.00	41.08 ± 0.70	41.00 ± 0.70
Prostate volume (ml)	13.15 ± 0.33	12.67 ± 0.42	12.81 ± 0.28	12.12 ± 0.61	12.33 ± 0.66	12.63 ± 0.32	12.10 ± 0.44	12.25 ± 0.62	12.67 ± 0.33
PSA (ng/ml)	0.95 ± 0.12	1.17 ± 0.08	1.16 ± 0.11	1.12 ± 0.26	1.21 ± 0.07	2.14 ± 0.94	0.98 ± 0.18	1.06 ± 0.07	0.91 ± 0.07

CHO, Cholesterol; LDL, low-density lipoprotein; TRIG, triglycerides; ALT, alanine amino-transferase; BUN, blood urea; Hb, hemoglobin; BW, body weight.
[a] $P < 0.05$ *vs.* control period.

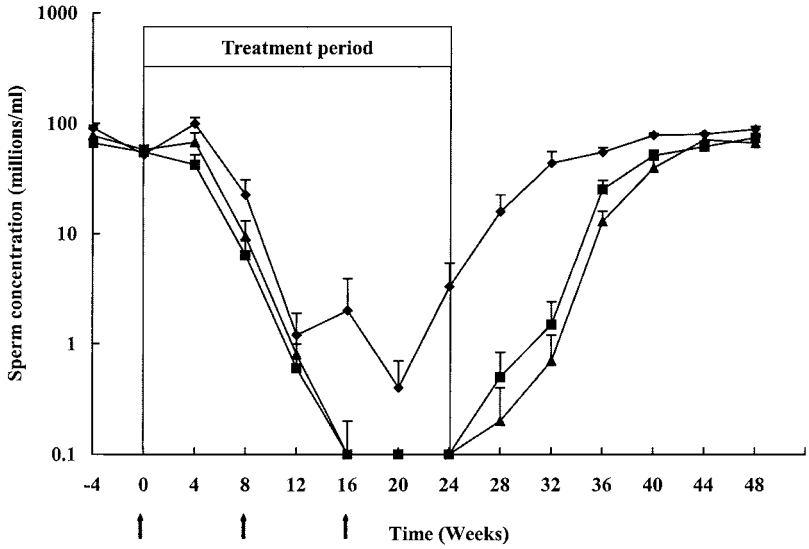

FIG. 1. Mean sperm concentrations in each group after TU alone or TU combined with DMPA at 8-wk injection intervals. Values are expressed as the mean ± SEM. *Arrows* indicate hormone injections. The y-axis is represented as a log scale. ♦, Group A; ■, group B; ▲, group C.

TABLE 2. Time and distribution of azoospermia (Azoo.) and severe oligozoospermia (Oligo.)

Week	1000 mg TU			1000 mg TU + 150 mg DMPA			1000 mg TU + 300 mg DMPA		
	Azoo.	Oligo.[a]	Oligo.[b]	Azoo.	Oligo.[a]	Oligo.[b]	Azoo.	Oligo.[a]	Oligo.[b]
4	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
8	0/10	2/10	0/10	0/10	3/10	1/10	0/10	2/10	1/10
12	7/10	0/10	1/10	8/10	2/10	0/10	7/10	2/10	0/10
16	8/10	0/10	1/10	10/10	0/10	0/10	9/10	0/10	1/10
20	8/10	1/10	1/10	10/10	0/10	0/10	10/10	0/10	0/10
24	7/10	1/10	0/10	10/10	0/10	0/10	10/10	0/10	0/10

[a] Sperm concentrations between 1.1 and 3 million/ml.
[b] Sperm concentrations 1 million/ml or less.

values at this time point were compared with baseline concentrations in each group. Sperm concentration as well as motility and morphology returned to baseline values or the normal reference ranges within the 24-wk recovery period in all volunteers, with the exception of one man in group C whose sperm concentration was 19×10^6/ml after 24 wk of recovery. An additional semen sample was obtained 4 wk later; the volunteer's sperm concentration had reached 56×10^6/ml, well over the reference value.

Reproductive hormones

Mean serum T concentrations were significantly increased over baseline values at 4 wk after the first injection in the TU-alone group and by the 12th wk of the treatment period in the TU/DMPA combination groups. Serum T concentrations were elevated at the 4th wk post injection throughout the course of the treatment period (Fig. 2A) but remained within the normal reference range. Mean serum T concentration at the 4th wk post injection in the TU-alone group was significantly higher when compared with both TU/DMPA combination groups. Serum T concentrations returned to baseline levels by the end of recovery period. An important finding in this study was that mean serum T concentrations dropped to below the normal reference range in both TU/DMPA combination groups during the initial 8 wk of the recovery period. Serum SHBG concentrations were slightly reduced by TU/DMPA administrations but not by TU alone. Mean serum-free T concentrations were significantly increased following the same pattern as serum total T levels (Fig. 2B), but there was no significant difference in the response among the three groups. The ratio of serum-free T to total T remained unchanged throughout the study.

Mean serum E_2 concentrations were also significantly increased following the same pattern as serum T concentrations, with levels over the upper limit of the normal reference range during the treatment period (Fig. 2C).

Significant suppression of both serum LH and FSH by the injections in each group was first demonstrated at wk 4; gonadotropin levels were maintained at very low levels throughout the treatment and into the recovery period and finally returned to their baselines at wk 48, the end of the recovery period (Fig. 3). In the TU-alone group, the regimen could not consistently and reliably suppress the gonadotropins throughout the 8-wk injection interval. Significant differences in the ability of the regimens to suppress gonadotropins were found between the TU-alone group and the TU/DMPA combination groups. There was no dose-response relationship between the degree of suppression of serum LH and FSH and the amount of DMPA administered.

Serum MPA concentrations were detectable in both groups B and C until wk 32. There was a clear dose-response relationship between the dose of DMPA administered and serum MPA concentrations (Fig. 4), which was evaluated by area under the serum MPA concentration-time curve. The area under the curve was 107.40 and 146.96 nmol/liter·wk for the low and high doses of DMPA, respectively ($P < 0.001$).

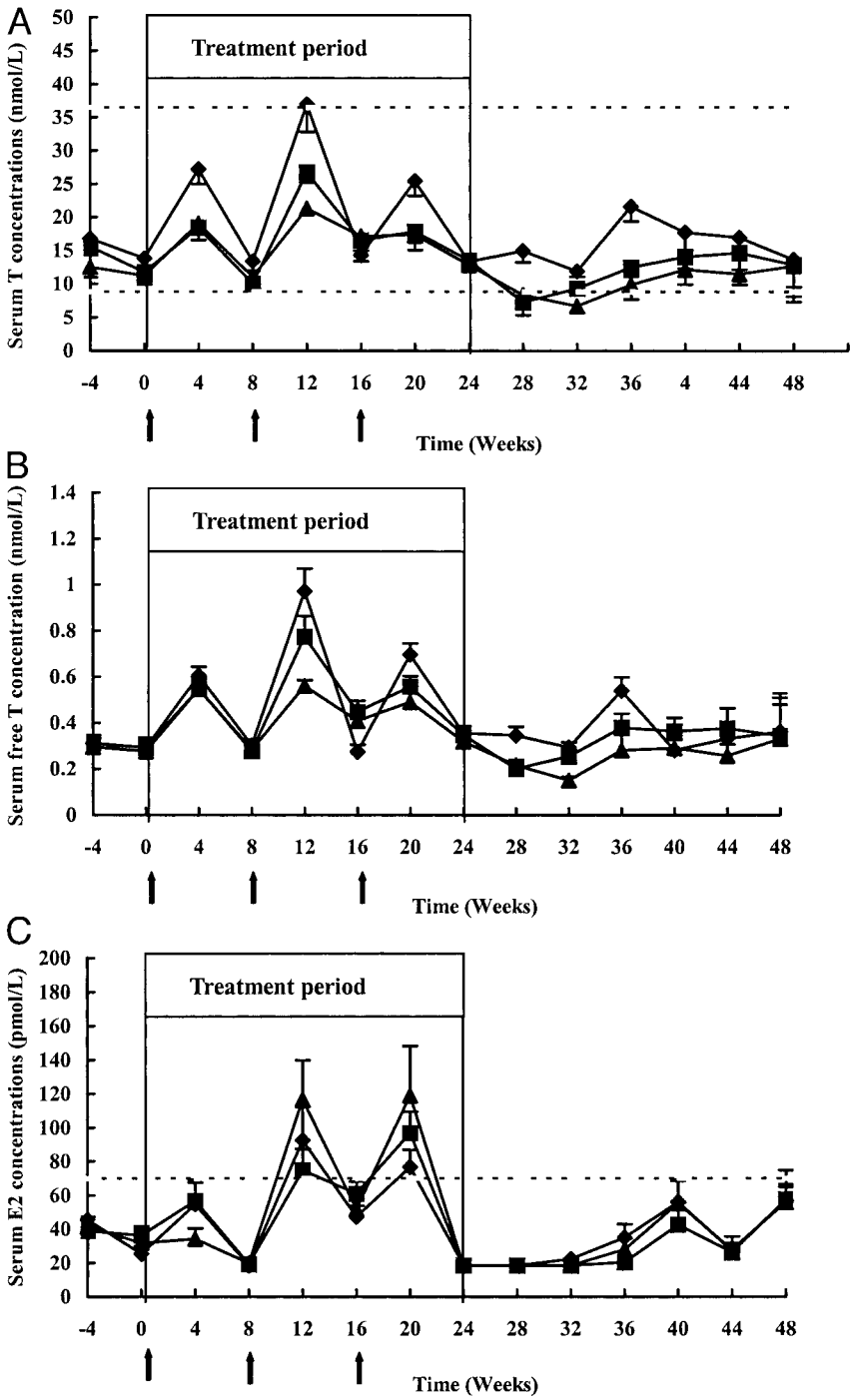

FIG. 2. Mean serum T (A), free T (B), and E_2 (C) concentrations in each group after TU alone or TU combined with DMPA at 8-wk injection intervals. Values are expressed as the mean ± SEM. *Arrows* indicate hormone injections. *Dotted lines* represent the upper and lower limits (A) or upper limits (C) of the eugonadal reference range for adult Chinese men. ♦, Group A; ■, group B; ▲, group C.

Serum PSA levels remained stable and within the normal reference range throughout the study period for all groups.

Lipid, hematology, and clinical blood chemistry

Mean HDL cholesterol levels significantly decreased 18, 19, and 18% in groups A, B, and C, respectively, during the treatment period, compared with baseline values, but there was no significant difference among the three groups. There was a tendency for mean concentrations of total and low-density lipoprotein cholesterol and triglyceride to increase within the normal reference ranges. Hemoglobin and hematocrit levels rose in each group during the treatment period, compared with baseline values, with a significant increase in hemoglobin level noted only in the TU-alone group. The changes in all parameters mentioned above remained within the normal reference ranges (Table 1). Lipid and hematology parameters all returned to baseline values during the recovery period. Blood chemistry assessments of liver and kidney functions, determined by alanine aminotransferase and blood urea measurements, remained within

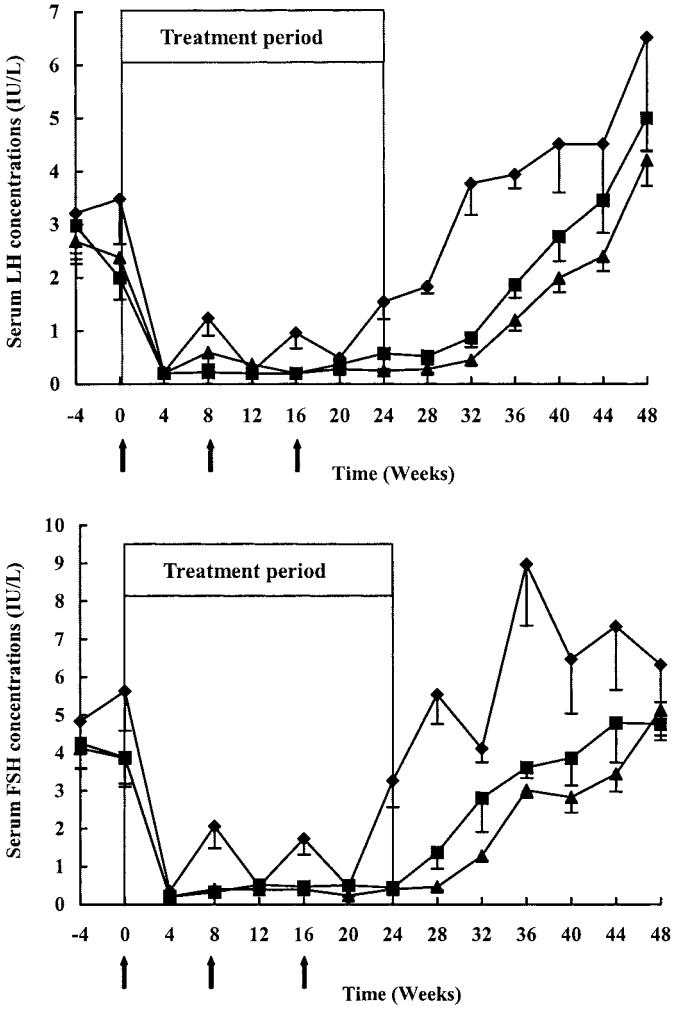

FIG. 3. Mean serum LH (A) and FSH (B) concentrations in each group after TU alone or TU combined with DMPA at 8-wk injection intervals. Values are expressed as the mean ± SEM. *Arrows* indicate hormone injections. ◆, Group A; ■, group B; ▲, group C.

the normal reference ranges throughout the study for all groups (Table 1).

Discussion

This report describes a Phase I clinical study of TU in combination with DMPA as a potential method of male hormonal contraception, with primary outcomes being suppression of spermatogenesis and reversibility. There is an obvious trend in the mean length of time required for the onset of spermatogenic suppression that was shorter in the TU/DMPA combination groups than in the TU-alone group. The lack of statistical significance may be attributable to the limitations induced by the small numbers of subjects in the study.

The administration of 1000 mg TU, without the addition of DMPA, at 8-wk injection intervals failed to sustain suppression of spermatogenesis in Chinese men in a previous study (23) and also in this study, presumably due to the failure of this regimen to reliably maintain inhibition of serum LH and FSH. In the current study, both combination regimens effectively and consistently suppressed sperm concentrations to infertile levels; however, recovery of spermatogenesis was slower in the combination groups, compared with the TU-alone group. This could be attributed to consistently high serum MPA concentrations, even in the early phase of the recovery period due to storage and release of MPA in fat tissue. This phenomenon of long-acting suppressive effects of MPA suggests that the progestin could be combined with an even longer-acting androgen preparation to increase the administration interval to up to 3 months during the treatment phase; this may enhance the acceptability of such a combination regimen for male hormonal contraception. These data also suggest that withdrawal of exogenous androgen in the recovery period could lead to androgen deficiency in some men due to the long-acting suppressive effects of MPA. Based on the efficacy and safety data from this study, the lower-dose combination of DMPA plus TU is recommended for further testing in an expanded clinical trial.

In the WHO multicenter study evaluating the effectiveness of TE-induced oligozoospermia and azoospermia for contraception, the pregnancy rates were directly related to the sperm concentrations (6). The WHO trial analysis defined the threshold of efficacy at 3×10^6/ml because this degree of suppression resulted in acceptable contraceptive efficacy rates. Therefore, a sperm concentration of 3×10^6/ml or less was defined as severe oligozoospermia for the purpose of the present study. However, in the WHO study, four pregnancies were diagnosed during the efficacy period when sperm density fell between 0.1 and 3×10^6/ml (6). When the failure rate was further analyzed among those men with severe oligozoospermia ($<3 \times 10^6$/ml) during the efficacy phase in a previous study evaluating 500 mg TU alone, it was almost 2-fold higher in men with sperm concentrations between 1.1 and 3×10^6/ml than in men with sperm concentrations between 0.1 and 1×10^6/ml during the early stage of the efficacy phase. In addition, sperm rebound during the efficacy phase was highly correlated with sperm concentrations between 1.1 and 3×10^6/ml (9). For these reasons, the threshold for severe oligozoospermia should be set at 1×10^6/ml for the purpose of contraceptive efficacy trials because this level is compatible with a high degree of contraceptive effectiveness (9). Similarly, in 2002 the investigators at the Sixth Summit Meeting on Hormonal Male Contraception reached a consensus on this threshold and recommended that a sperm concentration of 1 million/ml or less would be accepted as a surrogate parameter of spermatogenic suppression and therefore as the main outcome measure in Phase II clinical studies (24).

Serum T concentrations in all three treatment groups were maintained at or above pretreatment levels during the treatment period, even at their nadir. However, serum T concentrations measured 4 wk after each injection in the TU-alone group reached the upper limit of the normal threshold. Based on the results from a pharmacokinetic study of TU in tea seed oil (10), the time to reach maximum concentration is 2.6 d, and serum T concentrations can be maintained above the upper limit of normal range for 2–3 wk. Presumably, serum T concentrations in the TU-alone group might rise into the supraphysiological range in the intervening time between

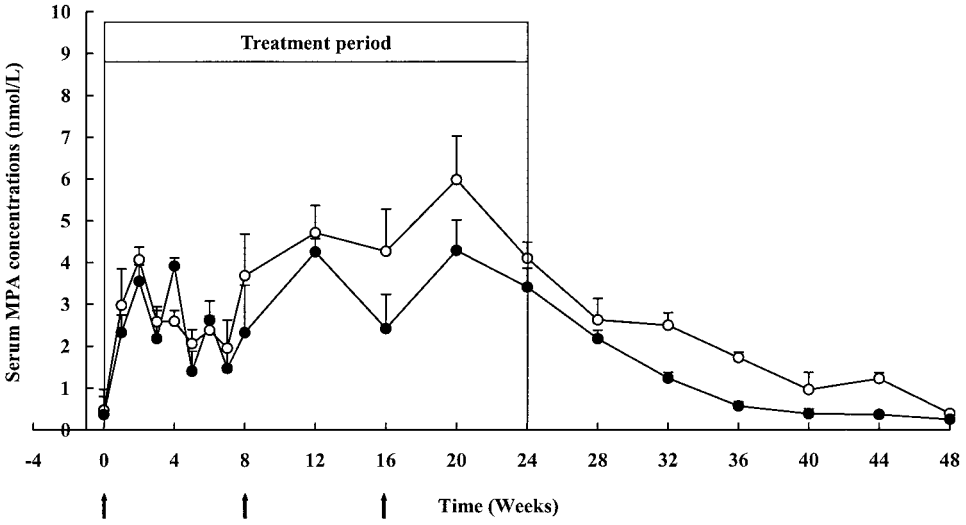

FIG. 4. Mean serum MPA concentrations in two combination groups after TU combined with DMPA at 8-wk injection intervals. Values are expressed as the mean ± SEM. *Closed* and *open symbols* represent groups B and C, respectively. *Arrows* indicate hormone injections.

the TU injection and blood measurement at 4 wk after each injection. Of considerable interest in our study is the observation that the mean serum T concentrations at the intermediate stage of an 8-wk injection interval in the TU-alone group were significantly higher when compared with each TU/DMPA combination group. One explanation for this result is that the combination of TU and DMPA induced a more profound inhibition of gonadotropins and endogenous T levels, as evidenced by the suppression of serum LH and FSH, and spermatogenesis. In addition, MPA has been reported to interfere with androgen biosynthesis and action at the testis (25, 26), which was indicated by lower serum T in the combination groups in the early stage of the recovery period. Therefore, endogenous serum T in the combination groups might be less than that in the TU-alone group. Another possibility is that MPA might have induced a more rapid clearance mechanism for serum T. It has been established that there is an inverse relationship between serum SHBG levels and the metabolic clearance rate of serum T (27). Thus, serum T concentrations may have decreased in combination groups as a result of increased metabolic clearance rate of the hormone. This hypothesis is supported by the findings that serum SHBG concentrations were reduced by TU/DMPA administration.

In the present study, the mean total testicular volume did not significantly change in any of the treatment groups. A possible reason for this is the relatively short treatment period (6 months), although in a previous similar study (23), the mean total testicular volume was decreased about 2 ml in Chinese men during the 6-month suppression phase. Another possibility might be the rather imprecise estimation of testicular volume by Prader's orchidometers. Clearly, use of ultrasonography would be preferable in measuring testicular volume in future clinical studies.

Tendencies toward weight gain and increased hemoglobin and hematocrit levels were found to result from all regimens evaluated in this study. The maximal increment in body weight during the treatment period was not as remarkable as that found in a previous clinical study (9) due to the relatively short treatment duration. Because the peak serum T levels were not monitored in this study, the serum T levels that were measured during the treatment period underestimated the maximal T levels to which the volunteers were exposed. Thus, intermediate T levels may have reached supraphysiological levels and prompted an increase in body weight and hemoglobin or hematocrit levels. Such disadvantages of this TU preparation highlight the need for the development of a long-acting androgen preparation with more stable delivery kinetics that could provide physiological levels of serum T when administered as part of a combination regimen. Recently injectable TU has been further developed, and a greater depot effect has been achieved by using castor oil instead of tea seed oil as vehicle. This preparation allows for longer injection intervals in the treatment of hypogonadism (28) and in contraceptive trials, when combined with a progestin, norethisterone enanthate (29, 30). Certainly a TU formulation with longer-acting and more stable pharmacokinetics would be of some advantages in further contraceptive trials in China.

Gynecomastia was diagnosed in two volunteers in the early stage of the recovery period; this effect has been reported in at least one previous male hormonal contraception study (18). Relatively higher serum E_2 levels at that time and responsiveness to antiestrogen therapy in these two men suggested that their gynecomastia was induced by elevated concentrations of serum E_2. These two men were randomized to the combination groups, in which serum E_2 levels were relatively higher but serum T levels were relatively lower during the late stage of the treatment period and early stage of recovery period, compared with the TU-alone group. It is hypothesized that gynecomastia may be related to a disorder of the proportion between serum T and E_2 levels caused by the altered absorption, distribution, and metabolism of TU when combined with DMPA administration.

It has been reported that regular administration of TE can cause mild suppression of HDL cholesterol in Caucasian men (31) as can the administration of TU in Chinese men (9). Similarly, in the present study, HDL cholesterol was decreased after administration of the combination regimens during the treatment period, when compared with baseline values. A chronic lowering of the HDL concentration, particularly in an individual with low baseline levels, may be

associated with an increased risk of coronary artery disease (32). This decrease in HDL cholesterol during the treatment period may also be a result of the presumably supraphysiological peak serum T concentrations in the present study, compared with more physiological and less fluctuating T levels produced by T implants or testosterone buciclate injections, which do not lead to decreased HDL cholesterol levels in healthy Caucasian men (3, 12).

Pharmacokinetic studies of DMPA in women show that this progestin has a prolonged duration of action due to its slow release from the tissue. MPA is detected in the serum within 30 min after an injection of 150 mg of the depot formulation. Serum MPA concentrations are generally maintained at a plateau for about 3 months, after which there is a gradual decline. Although a literature search has not revealed any publication regarding the pharmacokinetics of DMPA in men, the results of one clinical study implied that a single injection of DMPA at a dose of 150 mg has a long duration of effect (3 months) on patients with benign prostatic hyperplasia (33). Another clinical trial evaluating 800 mg of a fused crystalline T implant in combination with a single injection of 300 mg DMPA for application as a male contraceptive demonstrated that the combined regimen could significantly suppress gonadotropin levels and spermatogenesis for 3 months without serious side effects (12). In that study, a single injection of 300 mg DMPA maintained elevated serum MPA levels for up to 6 months (D. J. Handelsman, personal communication). The present study confirmed that administration of either 150 mg or 300 mg DMPA could maintain detectable levels of serum MPA for at least 4 months. Therefore, 150 mg DMPA, in combination with TU or a more long-acting androgen, could provide an optimal regimen to be administered at 2- to 3-month injection intervals for male contraception.

In summary, TU plus DMPA, at the tested dose combinations and administered at 8-wk injection intervals, can effectively suppress spermatogenesis to azoospermia in Chinese men without serious adverse effects. Because there was no difference in the efficacy of the two combination regimens tested and to reduce the total drug load, the lower-dose combination is recommended for testing in an expanded clinical trial or contraceptive efficacy study.

Acknowledgments

We are grateful to the Zhejiang Xian Ju Pharmaceutical Corp. for providing the injectable TU preparation. We thank Drs. Xiao-wei Liang and Wen-hong Lu and Zheng-yan Ge for their excellent technical assistance in hormone assays and biochemistry measurements. We gratefully acknowledge the help of Mr. Jing Dong with performing the statistical analysis and Ms. Jie Cong with data collection and monitoring. We also gratefully acknowledge the Contraceptive Research and Development Program for providing the research grant and Professor Jean M. Kaufman for kindly providing the free T calculator. We also gratefully acknowledge Drs. Kirsten Vogelsong and Doug Colvard for reviewing and commenting on a previous version of this manuscript.

Received July 28, 2003. Accepted February 3, 2004.

Address all correspondence and requests for reprints to: Yi-Qun Gu, M.D., Associate Professor, Department of Male Clinic Research, National Research Institute for Family Planning, Beijing 100081, China. E-mail: ygu90@hotmail.com.

This work was supported by the Contraceptive Research and Development Program (Arlington, VA) Project CIG-00-52 and Xianju Pharmaceutical Co. Ltd., Zhejiang, People's Republic of China.

References

1. **Paulsen CA, Leonard JM, Burges EC, Ospina LF** 1978 Male contraceptive development; re-examination of testosterone enanthate as an effective single entity agent. In: Patanelli DJ, ed. Hormonal control of male fertility, Bethesda, MD: U.S. Department of Health, Education, and Welfare (National Institutes of Health); 17–40
2. **Swerdloff RS, Palacios A, McClure RD, Campfield LA, Brosmen SA** 1978 Clinical evaluation of testosterone enanthate in the reversible suppression of spermatogenesis in the human male: efficacy, mechanism of action, and adverse effects. In: Patanelli DJ, ed. Hormonal control of male fertility. Bethesda, MD: U.S. Department of Health, Education, and Welfare (National Institutes of Health); 41–70
3. **Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E** 1995 Potential of testosterone buciclate for male contraception: endocrine differences between responders and nonresponders. J Clin Endocrinol Metab 80:2394–2403
4. **Wu FCW, Farley TMM, Peregoudov A, Waites GMH, World Health Organization Task Force for the Regulation of Male Fertility** 1996 Effects of testosterone enanthate in normal men: experience from a multicenter contraceptive efficacy study. Fertil Steril 65:626–636
5. **World Health Organization Task Force for the Regulation of Male Fertility** 1990 Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet. 336:955–959
6. **World Health Organization Task Force for the Regulation of Male Fertility** 1996 Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril 65:821–829
7. **Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ** 1999 A clinical trial of injectable testosterone undecanoate as a potential male contraceptive in normal Chinese men. J Clin Endocrinol Metab 84:3642–3647
8. **Wang CCL** 1999 Clinical studies using androgen alone for male contraceptive development. In: Rajalakshmi M, Griffin PD, eds. Male contraception: present and future. New Delhi, India: New Age International Publishers; 189–200
9. **Gu YQ, Wang XH, Xu D, Peng L, Cheng LF, Huang MK, Huang ZJ, Zhang GY** 2003 A multicenter contraceptive efficacy study of injectable testosterone undecanoate in healthy Chinese men. J Clin Endocrinol Metab 88:562–568
10. **Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ** 1998 A pharmacokinetic study of injectable testosterone undecanoate in hypogonadal men. J Androl 19:761–768
11. **Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM** 1996 Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. J Clin Endocrinol Metab 81:757–762
12. **Handelsman DJ, Conway AJ, Howe CJ, Turner L, Mackey MA** 1996 Establishing the minimum effective dose and additive effects of depot progestin in suppression of human spermatogenesis by a testosterone depot. J Clin Endocrinol Metab 81:4113–4121
13. **Meriggiola MC, Bremner WJ** 1997 Progestin-androgen combination regimens for male contraception. J Androl 18:240–244
14. **Anawalt BD, Bebb RA, Bremner WJ, Matsumoto AM** 1999 A lower dosage levonorgestrel and testosterone combination effectively suppresses spermatogenesis and circulating gonadotropin levels with fewer metabolic effects than higher dosage combinations. J Androl 20:407–414
15. **Melo JF, Coutinho EM** 1977 Inhibition of spermatogenesis in men with monthly injection of medroxyprogesterone acetate and testosterone enanthate. Contraception 15:627–634
16. **Alverez-Sanchez F, Faundes A, Brache V, Leon P** 1977 Attainment and maintenance of azoospermia with combined monthly injection of depot medroxyprogesterone acetate and testosterone enanthate. Contraception 15:635–648
17. **Brenner PF, Mishell DR, Bernstein GS, Ortiz A** 1977 Study of medroxyprogesterone acetate and testosterone enanthate as a male contraceptive. Contraception 15:679–691
18. **Chen ZD, Cai SL, Chen YZ, Jiang RG, Zhao RW, Gu WZ, Fang RY, Zhang YP** 1986 A clinical study of spermatogenesis suppression with combination of medroxyprogesterone acetate and testosterone undecanoate. Clin J Androl (Chinese) 1:18–20
19. **Moeloek N, Pujianto DA, Agustin R, Arsyad KM, Waluyo P, Priyugiharta Y, Mbizvo MT,** Achieving azoospermia by injections of testosterone undecanoate alone or combined with depot medroxyprogesterone acetate in Indonesian men (Jakarta center study). Proc 7th International Congress of Andrology, Montreal, Canada, 2001, Poster 1/2-133
20. **World Health Organization** 1992 WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge, UK: Cambridge University Press
21. **Zhang QS** 2001 Measurements of prostate volume. In: Zhang QS, Guo YL, eds. Ultrasonographic diagnosis and therapeutics of urinary system. Beijing: Scientific Literature Publishing House; 292–294

22. **Vermeulen A, Verdonck L, Kaufman JM** 1999 A clinical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672
23. **Gu YQ, Tong JS, Ma TZ, Wang XH** 2002 A dose-finding study of androgen combined with progestin for spermatogenic suppression in Chinese men. J Reprod Med (Chinese) 11:264–268
24. 2002 Announcement. Sixth Summit Meeting Consensus (Petersberg, Germany): Recommendation for Regulatory Approval for Hormonal Male Contraception. Int J Androl 25:375
25. **Satyawaroop PG, Gurpide E** 1978 A direct effect of medroxyprogesterone acetate on 17β-hydroxysteroid-dehydrogenase in adult rat testis. Endocrinology 102:1761–1765
26. **Worgul T, Baker HWG, Murry FT, Jefferson LS, Bardin CW** 1979 Direct effect of medroxyprogesterone acetate on the testis: possible mechanisms examined by testicular perfusion. Int J Androl 2:408–418
27. **Petra PH, Stanczyk FZ, Namkung PC, Fritz MA, Novy MJ** 1985 Direct effect of sex steroid-binding protein (SBP) of plasma on the metabolic clearance rate of testosterone in the rhesus macaque. J Steroid Biochem 22:739–746
28. **Nieschlag E, Buchter D, Von Eckardstein S, Abshagen K, Simoni M, Behre HM** 1999 Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol (Oxf) 51:757–763
29. **Kamischke A, Venherm S, Ploger D, von Eckardstein S, Nieschlag E** 2001 Intramuscular testosterone undecanoate and norethisterone enanthate in a clinical trial for male contraception. J Clin Endocrinol Metab 86:303–309
30. **Kamischke A, Heuermann T, Kruger K, von Eckardstein S, Schellschmidt I, Rubig A, Nieschlag E** 2002 An effective hormonal male contraceptive using testosterone undecanoate with oral or injectable norethisterone preparations. J Clin Endocrinol Metab 87:530–539
31. **Meriggiola MC, Marcovina S, Paulsen CA, Bremner WJ** 1995 Testosterone enanthate at a dose of 200 mg/week decrease HDL cholesterol levels in healthy men. Int J Androl 18:237–242
32. **Bagatell CJ, Heiman JR, Matsumoto AM, Rivier JE, Bremner WJ** 1994 Metabolic and behavioral effects of high-dose, exogenous testosterone in healthy men. J Clin Endocrinol Metab 79:561–567
33. **Onu PE** 1995 Depot medroxyprogesterone in the management of benign prostatic hyperplasia. Eur Urol 28:229–235

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.