Transient Scrotal Hyperthermia and Levonorgestrel Enhance Testosterone-Induced Spermatogenesis Suppression in Men through Increased Germ Cell Apoptosis

Christina Wang, Yu-Gui Cui, Xing-Hai Wang, Yue Jia, Amiya Sinha Hikim, Yan-He Lue, Jian-Son Tong, Li-Xin Qian,† Jia-Hao Sha, Zuo-Min Zhou, Laura Hull, Andrew Leung, and Ronald S. Swerdloff

Division of Endocrinology (C.W., Y.J., A.S.H., Y.-H.L., L.H., A.L., R.S.S.), Department of Medicine, Harbor-University of California, Los Angeles, Medical Center and the Los Angeles Biomedical Research Institute, Torrance, California 90509; Clinical Center of Reproductive Medicine, First Affiliated Hospital (Y.-G.C., Y.J., L.-X.Q.) and Key Laboratory of Reproductive Medicine (J.-H.S., Z.-M.Z.), Nanjing Medical University, Nanjing 210029, China; and Jiangsu Family Planning Research Institute (X.-H.W., J.-S.T.), Nanjing 210029, China

Context: In rodents and monkeys, a combination of hormonal and physical agents accelerates germ cell death.

Objective: A "proof of concept" study was performed to investigate whether addition of heat exposure or a progestin to an androgen induces germ cell death and more complete and rapid spermatogenesis suppression.

Design and Settings: A randomized clinical trial was performed at academic medical centers.

Participants: We treated four groups of healthy male volunteers (18 per group) for 18 wk: 1) testosterone undecanoate (TU) 1000 mg im (first dose), followed by 500 mg im every 6 wk; 2) submersion of scrota at 43 C in water for 30 min/d for 6 consecutive days; 3) TU plus heat; and 4) TU plus oral levonorgestrel (LNG) 250 μg/d.

Main Outcome Measures: Semen parameters, testicular histology, and germ cell apoptosis were the main outcome measures.

Results: Heat alone and TU plus heat suppressed sperm counts more than TU alone by wk 6. By wk 9, recovery began in the heat only group, whereas spermatogenesis remained suppressed in the TU plus heat group. Oral LNG plus TU suppressed spermatogenesis earlier and more severely than TU alone. At wk 2, significantly greater germ cell apoptosis occurred in heat and heat plus TU subjects, but not in subjects without heat treatment, compared with pretreatment subjects. By wk 9, markedly smaller seminiferous tubule diameters and fewer spermatocytes and spermatids were noted in all 12 biopsies from men receiving TU, TU plus LNG, with most dramatic differences for the TU plus heat group, whereas no differences from pretreatment biopsies were observed in men who received heat treatment only.

Conclusions: Heat causes a rapid and transient suppression of spermatogenesis. TU plus heat resulted in low-sperm output that was maintained by continuous treatment with TU. Addition of an oral progestin accelerated spermatogenesis suppression by TU alone. Increased germ cell apoptosis contributed to suppression of spermatogenesis. (J Clin Endocrinol Metab 92: 3292–3304, 2007)
22. In this proof of concept study, we address the question whether addition of a second “hit” such as a physical agent (heat) or another gonadotropin suppressing agent [a progestin, e.g., levonorgestrel (LNG)] to exogenous T treatment (first “hit”) will result in accelerated apoptosis, resulting in rapid and increased spermatogenesis suppression. As a secondary objective, results not discussed in this report, we studied the alterations in gene expression of regulators of spermatogenesis at various time points after intervention to identify signaling pathways that may provide novel targets responsive to the “two hits” that can be used in future male contraceptive development.

Subjects and Methods

Subjects

A total of 72 healthy Chinese male volunteers between the ages of 27 and 48 yr (35.6 ± 5.4 [mean ± sd]) were recruited from a glass/lens factory at the Nanjing Center. The subjects had no significant medical history, and all had a normal physical examination during recruitment. None of the subjects was undernourished. They had normal baseline hematopathy, blood biochemistry, urinalysis, fasting lipid profile, and three consecutive normal semen analysis at 2-wk intervals (sperm concentration > 20 million/ml; motility > 50%; and oval forms > 10%) according to the WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (23).

Study design

After a pretreatment observation phase of 4 wk, the eligible subjects were randomized in a 1:1:1:1 allocation to four groups for a treatment phase of 18 wk: group 1, testosterone undecanoate (TU); group 2, heat alone; group 3, TU plus heat; and group 4, TU plus LNG group. After randomization, treatment assignments were known to subjects and clinical staff, but not to laboratory and histological evaluators. On the first day of the treatment phase, 1000 mg loading dose followed by 500 mg TU (125 mg/ml in tea seed oil) was obtained from Xian-Ju Pharmaceutical Company (Zhejiang, People’s Republic of China), and was used in prior studies in China in hypogonadal men and male contraceptive trials (25–28). TU was administered as 8 ml (4 ml each in each buttock) im on injection on d 1, and then 500 mg (2 ml) at wk 6 and 12 by the study physician. The rationale for this dose regimen was based on prior studies of the Nanjing group, in which 1000 mg loading dose followed by 500 mg TU im every 4 wk resulted in azospermia (sperm concentration < 106/ml) in most healthy volunteers, and spacing of 500 mg TU injections every 6 wk led to incomplete suppression of spermatogenesis. Because our goal was to investigate potential additive effects of heat or progestin treatment on androgen alone treatment, we intentionally used longer intervals between TU injections to produce partial (incomplete) suppression of spermatogenesis.

LNG tablets containing 750 μg LNG (manufactured for emergency contraception in China) were obtained from Beijing Zi-Zhu Pharmacy Limited Company (Beijing, China). The tablet was divided into three parts (250 μg each, weighed by a balance), and the subjects took the three portions of the tablet on 3 consecutive days for 18 wk. The dose of LNG chosen was based on prior studies in which this dose of LNG achieved maximum suppression of spermatogenesis when combined with a suboptimal dose of T enanthate injection (29).

Heat treatment was performed under supervision to ensure compliance and careful monitoring of adverse effects. To limit the effect of heat to the testes and other parts of the body, the scrotum of each subject was submerged into a warm water bath in which the temperature of the water was gradually increased and maintained at 43°C. Water and scrotal temperatures were simultaneously measured with two thermometer probes with appropriate insulation pads on the skin. When the temperature of the skin reached 43°C, the participant remained sitting in the water bath for 30 min/d for 6 consecutive days. The heat treatment regimen was then repeated for 3 additional cycles for a total of 18 wk. The rationale for this dose regimen was based on prior studies of scrotal immersion in men (30–33). The scrota were examined immediately after heating that showed no scrotal skin changes.

Open biopsy of the one testis was performed under local anesthesia under the supervision of an experienced surgeon (L.-X.Q.), who routinely performed this procedure for the investigation of male infertility in the Nanjing Institute. Open biopsy was performed instead of fine needle biopsy to ensure that good quality testicular tissues were obtained for the studies and to control risk of bleeding (34, 35). After local anesthesia, a 5-mm incision was made through the skin, tunica vaginalis, and tunica albuginea, and a small piece of tissue up to 100 mg was obtained and divided into three portions: one fixed in Bouin solution for apoptosis assessment, another frozen in liquid nitrogen for protein analyses, and the third stored in RNA Later (Ambion, Inc., Austin, TX) for gene expression studies. Subjects were given scrotal support and asked not to engage in strenuous activity after the biopsy. Before the study, the risks of testicular biopsy, including pain, hemorrhage, and infection, were explained to the participants. Because of these possible risks of the testicular biopsy, only men who had completed their family were offered the testicular biopsy studies.

Hormone assays and semen analyses

All serum samples of the subjects were measured in validated hormone assays (36–39) in the Los Angeles center. Serum T levels were assayed by a specific RIA (coat-a-tube; Diagnostic Products Corp., Los Angeles, CA). To control for sensitivity and accuracy of the assays, 10% of the samples were run in duplicate. Intraassay coefficients of variation were < 10% for all analytes, and interassay coefficients of variation were < 15% for all analytes except LH, which had a variability of < 20%.

Semen and Sperm-Cervical Mucus Interaction
of IgG and subsequently incubated with a 1:100 dilution of a polyclonal anti-Vasa (DDX4) antibody (Abcam, Cambridge, MA). Immunoreactivity was detected using biotinylated antimouse IgG secondary antibody, followed by avidin-biotinylated horse radish peroxidase complex visualized with diaminobenzidine tetrahydrochloride, as per the manufacturer’s instructions (UniTect 228 ABC Immunohistochemistry Detection System; Calbiochem, La Jolla, CA). Slides were counter-stained with hematoxylin and reviewed with a Zeiss Axioskop 40 microscope (Zeiss, Thornwood, NY).

Statistical analyses

The four randomized treatment groups were compared for age, body weight, body mass index (BMI), sperm concentration, T, LH, and FSH at baseline before treatment using the nonparametric Kruskal-Wallis tests, due to skewness in the distributions of some of these measures. Changes over time in sperm concentration did not display skewness, and, thus, mean changes within treatment groups and differences in these changes among treatment groups were analyzed with repeated measures ANOVA, implemented with maximum likelihood estimation using unstructured covariance. Sperm motility and morphology and serum hormones were separately analyzed using the same ANOVA models.

Sperm concentration was also analyzed according to percentages of subjects within each treatment group who were suppressed to oligozoospermia (<3 million/ml) or azoospermia (zero measured sperm) at each of the six sperm collection periods between wk 6 and 22. These percentages were compared among treatment groups using Fisher’s exact tests with Bonferroni adjustment for an overall 0.05 level of significance for multiple statistical comparisons for six collection periods.

Changes from baseline in complete blood count and clinical chemistry measures were averaged for each subject over all posttreatment measurement periods, and the significance of mean changes within treatment groups were calculated in one-way ANOVAs.

Mean AI at wk 2 for each treatment group was compared with that from biopsies at baseline, before treatment, using ANOVA with Dunnett’s correction for multiple comparisons with a control. The associations between AIs and sperm concentrations were measured with Pearson correlations.

Results

Subjects

There were no significant differences in age, weight, height, BMI, sperm concentration, serum levels of T, LH, and FSH among the subjects in the different treatment groups at baseline before treatment, as shown in Table 1.

Sperm concentration

Sperm concentrations at baseline (pretreatment), treatment, and recovery (posttreatment) phases are shown in Fig. 1 (upper panel). At 3 wk, sperm concentration decreased significantly in all groups except the TU alone group. Mean sperm concentration after heat treatment decreased maximally at 6 wk and then gradually returned to baseline levels.
FIG. 1. Mean sperm concentration in the volunteers in logarithmic scale (upper panel), sperm motility (middle panel), and sperm morphology (lower panel). Left panel shows data from participants who were treated with TU injections alone 1000 mg at d 1, and then 500 mg at wk 6 and 12 (closed circles, ●), heat alone applied from d 8–3 (open square, □), and combined TU plus heat (closed triangles, ▲). Right panel shows those treated with TU alone (closed circles, ●) and TU plus daily oral 250 µg/d LNG (open diamonds, ◆). Note that in this figure and in Figs. 3 and 4, the TU alone group consists of the same subjects in both the left and right panels.
by wk 12 (left panel). TU alone suppressed sperm output much more slowly and reached maximum suppression at wk 18, the end of the treatment period. The combination of heat plus TU injections suppressed mean sperm concentration markedly by 6 wk and then remained suppressed at low levels until the end of the treatment period. At both 3 and 6 wk, heat only and TU plus heat groups had suppressed mean sperm concentration significantly more than TU alone group ($P < 0.0001$), but these two groups receiving heat did not differ significantly. At 9 wk, TU plus heat and TU alone groups had further suppression, whereas the heat only group was already recovering. At 9 wk, the suppression of sperm concentration was significantly ($P < 0.0005$) less for each treatment alone than TU plus heat. At wk 3 and 6, TU plus LNG suppressed sperm concentration significantly greater than TU (same as the group in Fig. 1, left panel) alone ($P < 0.0001$), but less than TU plus heat ($P < 0.0001$). The combination of TU plus LNG continued to suppress spermatogenesis to a very low level until the end of treatment. During the recovery period, mean sperm concentration in all groups returned to levels above 20 million/ml by wk 30.

Of subjects that had sperm in the ejaculate, group differences in the mean percentages that were motile followed the same pattern as for sperm concentration (Fig. 1, middle panel). Abnormal morphology rates in ejaculated sperm were similar, with no significant group differences ($P = 0.24$) in changes in mean percent normal sperm from baseline to 6 wk, and similar ($P = 0.25$) near return to baseline levels at 30 wk (Fig. 1, lower panel).

### Proportion of subjects with severe oligozoospermia and azoospermia

No subject in the heat alone group had sperm concentration less than 3 million/ml at any time (Fig. 2). All subjects with sperm concentration less than 1 million/ml at any particular time reached azoospermia at that time, except one subject in the TU plus heat group at wk 18. Azoospermia was achieved at wk 18 by seven of 18, six of 18, and 17 of 18 men in the TU, TU plus heat, and TU plus LNG groups, respectively. Significantly ($P < 0.05$) more men achieved azoospermia: 1) in the TU plus LNG group compared with each of the
other groups at wk 12–22, 2) in the TU and TU plus heat group compared with the heat group at wk 18, and 3) in the TU plus heat group compared with the heat group at wk 15. In addition, the TU plus heat group had significantly more men achieving oligozoospermia compared with each of the other groups at wk 9.

**Serum hormone concentrations**

As shown in Fig. 3, upper panel, mean serum total T appeared to increase, but not significantly (\( P = 0.41 \)) after heat treatment alone but increased 3 wk after each injection in the TU alone, TU plus heat, and TU plus LNG groups, which did not differ from each other (\( P = 0.20 \)). Identical results were obtained for serum-free T levels (Fig. 3, middle panel). Mean serum SHBG level was unchanged in the heat only group but showed a small but significant decrease in the TU alone and TU plus heat groups, and a significantly greater reduction (to 45% of baseline levels) in the TU plus LNG group compared with the other TU groups (\( P < 0.002 \)) (Fig. 3, lower panel).

In synchrony with increases in serum total and free T after each injection, mean serum LH (Fig. 4, upper panel), and FSH (Fig. 4, middle panel) concentrations showed sig-
significant decreases 3 wk after each TU injection in the TU and TU plus heat groups ($P < 0.05$). The TU plus LNG group also showed significant mean LH and FSH decreases 3 wk after the first injection ($P < 0.0001$), which were maintained less than 0.5 IU/liter from wk 3–18, resulting in significantly ($P < 0.003$) lower mean concentrations than the other groups over that period. Mean serum inhibin (Fig. 4, lower panel) was not significantly changed in the heat or the TU group but decreased significantly in the TU plus heat group (by 15%; $P = 0.05$) and in the TU plus LNG group (by 34%; $P < 0.0001$).

**Safety monitoring of the subjects**

No adverse events were observed with transient testicular hyperthermia. Three subjects reported pain at the incision site, and a small hematoma was observed in another subject after testicular biopsy, which subsided in a few days without

---

**FIG. 4.** Mean serum LH (upper panel), FSH (middle panel), and inhibin (lower panel) levels in the four treatment groups (LH and FSH in logarithmic scale). *Left panel* shows data from participants who were treated with TU alone (closed circles, ●), heat alone (open square, □), and combined TU plus heat (closed triangles, ▼). *Right panel* shows those treated with TU alone (closed circles, ●) and TU plus LNG (open diamonds, ◆).
changes included reduction (26.2%) in tubular diameter, and a marked decrease in the number of spermatocytes and spermatids (Fig. 5B). The overall testicular morphology of the TU plus LNG (Fig. 5E) group was similar to that of the TU alone group (Fig. 5B) except for an additional decrease in germ cell numbers. The maximum impairments of spermatogenesis occurred in the TU plus H group. There was a further reduction in tubule diameter, and the morphological alterations were more extensive with complete obliteration of tubular lumen, thickened basal lamina, and complete absence of mature spermatids (Fig. 5D). Quantitative analysis further confirmed histological findings (Table 2) and revealed a marked decrease in tubule diameter (38.5%) and Vv% of the seminiferous tubules (31.7%) in comparison with controls. Vv% of tubular lumens declined markedly to only 3.3% of control values. The morphological appearance of the testes after 9 wk of heat alone was similar to that of the control group.

**Activation of germ cell apoptosis**

Quantitative assessment of germ cell apoptosis (Fig. 6, A–E) was performed in testicular biopsies after 2 wk of treatment, and the incidence of germ cell apoptosis (expressed as numbers per 100 Sertoli cells) in various treatment groups is summarized in Fig. 6F. At 2 wk, no significant differences in the incidence of apoptosis were noted between baseline and TU or TU plus LNG groups. In contrast, heat alone or in combination with TU resulted in a significant increase of germ cell apoptosis when compared with that at pretreatment, with no significant differences between these two groups. No attempts were made in the present study to quantitate the incidence of germ cell apoptosis after 9 wk of treatment because the results may not be meaningful due to: 1) rapid clearance of apoptotic germ cells through phagocytosis by Sertoli cells in severely regressed tubules in subjects treated with TU alone, or in combination with heat or LNG, and 2) the recovery of spermatogenesis in heat alone group. AI at 2 wk significantly correlated with the decrease in sperm concentration from baseline to 3 wk in the men providing these biopsies (P < 0.05), with this association weakening at 6 wk (P = 0.15) and disappearing at 9 wk (P > 0.99).

**Vasa expression and localization in human testes**

The expression of Vasa, a germ cell specific marker (43, 44), was consistent in all the biopsies from the same treatment.

### TABLE 2. Tubule diameter and volumetric composition of testes in men at baseline and after various treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubule diameter (µm)</th>
<th>Tubule volume (Vv%)</th>
<th>Lumen volume (Vv%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>183.3 ± 6.1</td>
<td>68.5 ± 2.7</td>
<td>15.1 ± 1.7</td>
</tr>
<tr>
<td>TU 2 wk</td>
<td>165.5 ± 9.6</td>
<td>68.4 ± 4.0</td>
<td>14.8 ± 3.0</td>
</tr>
<tr>
<td>Heat 2 wk</td>
<td>165.4 ± 6.2</td>
<td>66.7 ± 3.5</td>
<td>14.7 ± 1.5</td>
</tr>
<tr>
<td>TU + heat 2 wk</td>
<td>155.1 ± 4.2^a</td>
<td>70.2 ± 2.4</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>TU + LNG 2 wk</td>
<td>167.6 ± 6.8</td>
<td>72.7 ± 2.5</td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>TU 9 wk</td>
<td>139.0 ± 6.4^a</td>
<td>63.1 ± 6.4</td>
<td>15.0 ± 2.6</td>
</tr>
<tr>
<td>Heat 9 wk</td>
<td>186.3 ± 12.3</td>
<td>72.7 ± 0.8</td>
<td>13.5 ± 0.5</td>
</tr>
<tr>
<td>TU + heat 9 wk</td>
<td>115.7 ± 6.8^a</td>
<td>46.8 ± 1.0^a</td>
<td>0.5 ± 0.4^a</td>
</tr>
<tr>
<td>TU + LNG 9 wk</td>
<td>135.6 ± 4.5^a</td>
<td>57.2 ± 3.7</td>
<td>7.6 ± 1.7^a</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.  
^a P < 0.05 compared with baseline.
group. At 2 wk, Vasa staining was present in spermatocytes and spermatids, which appeared not different among the treatment groups (data not shown). Representative sections of testicular biopsy from each group after 9 wk of treatment showed Vasa localization mainly in spermatocytes and round spermatids, with moderate expression in spermatogonia at baseline and after heat treatment alone (Fig. 7). Marked loss of Vasa positive round spermatids was noted in the T alone group. Vasa positive spermatocytes and spermatids were lost in shrunken seminiferous tubules both in T plus heat and T plus LNG groups, however, spermatogonia remained in these combined treatment groups.

Discussion

In this study, we increased the interval between TU injections that was known to be suboptimal in the suppression of spermatogenesis in Chinese men (25) to allow us to examine potential additive effects of a physical agent such as heat or a progestin like LNG to enhance spermatogenesis by an androgen alone. The results of suppression of spermatogenesis in our study were inferior to those using a loading dose of 1000 mg, followed by 500 mg every 4 wk by design. The levels of T, and free T attained, and the suppression of LH, FSH, and SHBG mirrored those previously reported in Chinese men who were administered 500 or 1000 mg TU in tea seed oil (the same preparation used in this study) every 4 wk (27, 28) and our recent study of TU in castor oil given every 8 wk to non-Asian men (39). The suppression of spermatogenesis by TU alone was slow, and sperm output decreased to azoospermia and severe oligozoospermia only in 50% of subjects at the end of 18 wk of treatment. Our experimental paradigm of testicular warming was based on the previously published wet heating method (33). We found significant decreases in sperm concentration beginning at 3 wk and maximally at 6 wk, which then recovered to baseline by wk 12. These results are similar to the earlier testicular heating by testicular insulation (31, 32) or experimental transient artificial cryptorchidism (45, 46) in healthy volunteers. We also showed that mild transient elevation of testicular temperature in combination with TU significantly enhanced suppression of spermatogenesis by TU alone during the early treatment period between 3 and 9 wk, and heat alone from wk 9–18. Thus, we have confirmed that our observations previously demonstrated in rodents and monkeys (1, 2) were present in men using a similar experimental paradigm. Similar to other reports, we also showed in this study that addition of oral LNG to this suboptimal dosing interval of TU for suppression of spermatogenesis enhanced the effect of the androgen alone on decreasing sperm output throughout the treatment period (29, 36, 47, 48). This enhanced suppression was not accompanied by higher levels of serum total or free T but by more effective and complete suppression of serum gonadotropin levels in the LNG plus T group. Serum inhibin showed significant decreases in the TU combined with heat and LNG groups compared with the TU and heat alone groups, suggesting more severe Sertoli cell dysfunction, supporting our results of more suppression of spermatogenesis. Thus, in this proof of concept study in men, we showed that the combination of the “two hits,” exogenous TU (“hit 1”) and transient testicular hyperthermia (“hit 2”), resulted in accelerated suppression of spermatogenesis in the first few weeks compared with either treatment alone. In addition, we showed the enhancement of exogenous T (“hit 1”) suppression of spermatogenesis by LNG (“hit 2”), which had been demonstrated in many other studies (10, 11, 13, 38).

Histological sections of the testis at 2 wk showed no gross

![FIG. 5. A–E, Representative examples of spermatogenic alterations in various treatment groups after 9 wk of treatment. Compared with controls (A), striking differences in testicular morphology were noted in TU alone (B), or in combination with heat (D) or LNG (E). The maximum impairment of spermatogenesis was noted in the TU plus heat group. The morphological appearance of the testis after 9 wk of heat treatment (C) was essentially similar to that of the control group (A). Scale bar, 0.05 mm.]
FIG. 6. A–E, In situ detection of apoptotic germ cells (black arrow) in testicular biopsies from control (A), TU alone (B), heat alone (C), or TU in combination with heat (D) or LNG (E) after 2 wk of treatment. Apoptosis was detected by a terminal deoxynucleotidyl transferase-mediated deoxy-uridine 5’-triphosphate nick end labeling assay. Methyl green was used as a counterstain. Scale bar, 0.05 mm. F, Comparison of mean AI of germ cells among various treatment groups at 2 wk. Heat alone or in combination with TU resulted in significantly greater germ cell apoptosis when compared with that of controls. However, no significant differences in the incidence of apoptosis were noted between controls and TU or TU plus LNG groups. Values are the mean ± SEM. *P < 0.05 from ANOVA with Dunnett’s adjustment.
Fig. 7. Vasa (Mvh or DDX4) expression from control (A), TU alone (B), heat alone (C), TU plus heat (D), and TU plus LNG-treated (E) subjects. Vasa protein was localized in spermatocytes (black arrow) and round spermatids (red arrow). After 9 wk of treatment, Vasa expression was similar in control (A) and heat (C) alone group but was decreased in some tubules in the TU (B) alone group. Seminiferous tubules were shrunken with a loss of the majority of Vasa positive spermatocytes and round spermatids, but spermatogonia (yellow arrows) remained in the seminiferous tubules in TU plus heat (D) and TU plus LNG (E) treated groups. Scale bar, 0.05 mm.

differences among all treatment groups compared with the pretreatment biopsies, but morphometric analyses detected a modest (17.6%) decrease in tubule diameter in TU plus heat group compared with baseline values. This implies an additive effect of heat on TU during the early phase of suppression of spermatogenesis. At 9 wk, the combination groups (TU plus heat and TU plus LNG) showed a marked decrease in spermatogenesis compared with the T alone groups, whereas in the heat alone group, recovery of spermatogenesis began. This decrease was mainly in spermatocytes and spermatid, as confirmed by the expression of Vasa, a marker for premeiotic and postmeiotic sperm cells (43, 44). A novel finding was that a rapid and marked decrease in sperm concentrations, after mild transient testicular hyperthermia in combination with TU during the early treatment period between 3 and 9 wk, was associated with significant \((P < 0.05)\) activation of germ cell apoptosis within 2 wk of treatment. Our data constitute the first demonstration of the involvement of apoptosis in suppression of spermatogenesis in healthy men after heat plus TU treatment that is consistent with our earlier studies in rats (1) and monkeys (2), indicating that apoptosis is an important determinant of hormone and heat-induced suppression of spermatogenesis. No attempts were made in the present study to measure the rate of spermatogonia proliferation in these men under various treatment regimens. However, there are studies indicating that the B spermatogonia were the first germ cells to decrease after 2 wk of treatment with T enanthate alone or in combination with depot medroxyprogesterone acetate (17, 49). This implies that inhibition of spermatogonial proliferation is also an important determinant of sperm output after T and/or progestin treatment. Earlier studies by us in rodents (50, 51) as well as by others in primates, including men (17, 20), provided evidence indicating that the failure of spermatogenesis, characterized by the presence of elongated spermatids deep within the seminiferous epithelium, also plays an important role in the suppression of spermatogenesis after hormonal contraception. Unlike those reports, we did not observe failure of spermiogenesis in the present study after any of the treatment regimens. The reason for this difference is not known but could relate to testicular biopsies obtained at different time points after treatment with TU plus LNG compared with earlier studies. Moreover, the failure of spermiogenesis is a “dynamic” event, whereas testicular biopsy was taken at a “static” time point. The marked decrease in sperm concentration at 3 wk in the heat, TU plus heat, and TU plus LNG groups suggests that defects in spermiogenesis is likely. Ethnic differences in gonadal axis regulation, susceptibility of germ cells to apoptosis, or testicular morphology and spermatogenic potential between the Chinese and Caucasian men may also play a role (41, 52, 53). Thus, the mechanisms to explain the suppression of spermatogenesis under various treatment regimens include inhibition of spermatogonial proliferation, activation of germ cell apoptosis, and failure of sperm release.

Although scrotal warming adds to the efficacy of TU to suppress spermatogenesis earlier, our goal in this study was not to use the combined heat plus TU treatment as a practical, reversible method of male contraception but to investigate the mechanisms underlying the heightened spermatogenesis suppression with the combined intervention using an androgen with either heat or a progestin. We plan to define the key signal transduction pathways leading to accelerated apoptosis and decreased spermatogenesis by gene expression studies once our proof of concept study supported our hypothesis. A recent study (54) assessed selected testicular gene expression using quantitative PCR after treatment by a GnRH antagonist with T with or without the addition of desogestrel, a potent oral progestin. They demonstrated that the steroidogenic enzyme genes were markedly reduced,
and Sertoli cell genes such as inhibin α and the spermatocyte marker acrosin-binding protein were suppressed more in the group that received desogestrel in addition to the GnRH antagonist and T. In that study, testicular biopsy specimens were obtained at the end of the treatment period of 4 wk, gene profiling was not performed, and histological examination of the samples was not possible because the testicular biopsies were performed by needle biopsy (54). Thus, the study could have missed important known and unknown genes that may be the important regulators of germ cell proliferation, apoptosis, or spermiation early after initiation of treatment. Our experimental model will allow us to identify novel, testis-specific targets (key signaling pathways, specific kinases and phosphatases, and other druggable proteins) for the development of male contraceptive agents of the future. These new targets will not use hormones or heat but will act on master switches specific to the testis to turn spermatogenesis “on” or “off” for enhancement of male fertility or male fertility regulation.

Acknowledgments

We thank the General Clinical Research Center laboratory and Endocrine Research Laboratory at Harbor-University of California, Los Angeles, Medical Center/ Los Angeles Biomedical Research Institute for assisting with the measurement of the hormones.

Received February 16, 2007. Accepted May 8, 2007.

Address all correspondence and requests for reprints to: Christina Wang, M.D., General Clinical Research Center, 1000 West Carson Street, Torrance, California 90509. E-mail: wang@labiomed.org.

The study was supported by grants from the Contraceptive Research and Development Program and the Mellon Foundation (MFG-02-64, MFG-03-67), and the General Clinical Research Center at Harbor-University of California, Los Angeles, Medical Center (MO1-RR00425).

Disclosure Statement: The authors have nothing to declare in relation to materials presented in this manuscript.

References


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