Ketoconazole therapy: hormonal and clinical effects in non-tumoral hyperandrogenism

Antonio J Vidal-Puig$^1$, Manuel Muñoz-Torres$^1$, Esteban Jódar-Gimeno$^1$, Carlos J García-Calvente$^1$, P Lardelli$^1$, María E Ruiz-Requena$^1$ and Fernando Escobar-Jiménez$^1$

Department of Internal Medicine I (Endocrine Division)$^1$, Department of Epidemiology$^2$ and Department of Biochemistry$^3$, University of Granada Hospital, Granada, Spain


The aim of this study was to assess the usefulness of ketoconazole as a therapeutic alternative to polycystic ovary syndrome. The study group comprised 37 women with signs of hyperandrogenism (hirsutism, acne) and oligomenorrhea. A low dose (400 mg/day) of ketoconazole was tested in a 9-month prospective clinical study. Clinical response (Ferriman & Gallway score, acne) and modifications in hormone pattern (luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone, prolactin, 17-hydroxy-progesterone, androstenedione, steroid hormone-binding globulin, dehydroepiandrosterone sulfate, cortisol, adrenocorticotropic hormone (ACTH) and free testosterone index) were measured, and ACTH stimulation tests were performed. Tolerance and side-effects also were assessed. After 9 months of ketoconazole treatment, the patients' Ferriman & Gallway scores (18.26 ± 4.6 vs 12.4 ± 4.1; p < 0.001) and acne had improved markedly. Hormone patterns also became more favorable, with decreases in androgenic steroids (testosterone, androstenedione, free testosterone index and dehydroepiandrosterone sulfate: p < 0.01) and increases in estradiol (p < 0.01). Basal cortisol levels and cortisol after ACTH stimulation were not changed significantly, remaining within the reference range. Increases in ACTH were observed only in the 3rd month (p < 0.01). Initial levels of androgenic steroids were correlated inversely with their percentage decrease in successive samplings. Decreases in adrenal androgenic steroids were associated with an increase in steroid hormone-binding globulin. The side-effects of treatment, although not severe, caused some discomfort and led to a high drop-out rate (30%). Our results suggest that low doses of ketoconazole (400 mg/day) are an alternative therapy for non-tumoral hyperandrogenism, although the bothersome side-effects, which require close monitoring and maximal patient compliance, make this treatment advisable only in selected patients.

Manuel Muñoz-Torres. Plaza Isabel la Católica no. 2, E-18009, Granada, Spain

Ketoconazole (KTZ), an imidazole derivative able to inhibit cytochrome P-450-dependent enzymatic reactions (1), has been used to treat Cushing’s syndrome (2, 3), precocious puberty (4) and prostate carcinoma (5), because of its ability to inhibit gonadal and adrenal steroidogenesis (6).

The activity of KTZ is dose dependent, and the drug appears to inhibit selectively 17,20-desmolase and 17α-hydroxylase when given at doses of 400–600 mg/day (7). This feature makes KTZ a potentially useful agent in the treatment of non-tumoral hyperandrogenisms, such as polycystic ovarian syndrome (PCOS) or hyperthecosis (8, 9, 12).

The search for new modes of treatment for these disorders has been driven by the large number of patients with these problems, and by the fact that none of the therapeutic options currently available is entirely free of side-effects.

In this report we present the results of a clinical study of the effectiveness of low-dose KTZ treatment for hyperandrogenic conditions generally classified as PCOS (13, 14), and representing a variety of lesions at adrenal, ovarian and hypothalamic–pituitary sites. The study was designed to assess the hormonal and clinical response to treatment, and the possible complications and side-effects arising from 9 months’ treatment with KTZ at a dose of 400 mg/day.

Patients and methods

Patients

Thirty-seven women seen at the Endocrinology Division of the University of Granada Hospital (Granada, Spain) between May 1989 and April 1991 inclusive were
evaluated for hirsutism associated with an oligomenorrhea (cycle length of > 35 days) and occasionally acne (N = 11). The mean age of the sample was 21.3 years (range 16–29) and the mean body mass index was 23.0 ± 0.3. Cushing’s syndrome, androgen-secreting tumors, prolactinomas, hyper–hypothyroidism and 21α-hydroxylase deficits were excluded. None of the patients had taken any medication known to affect pituitary–gonadal function.

Study protocol
All patients were treated with two daily doses of 200 mg of KTZ (200 mg/12 h) for 9 months, and were followed for an additional month after treatment was stopped. Hirsutism scores were obtained for each patient before therapy was started, and after it was discontinued, using the method of Ferriman and Gallway (15). Changes in menstrual pattern were assessed by the number of menstruations during the 10 months prior to the study and during the 10 months of study. Acne was scored on a scale of four: 0 (none), 1 (mild), 2 (moderate) or 3 (severe).

Before treatment was started, hormonal studies of the follicular phase of the menstrual cycle were performed on days 3–8 in all women with regular menses. Serum concentrations of LH, FSH and ACTH were measured using immunoradiometric assays (CIS Bioindustries, GIF-sur-Yvette, France). Estradiol (E2), testosterone and prolactin (PRL) were measured with a commercial double-antibody radioimmunoassay (Sorin, Sallugia, Italy), and 17-hydroxy-progesterone (170HP) and androstenedione were measured with a non-extraction radioimmunoassay kit from Diagnostic Products Ltd. (Los Angeles, USA). Steroid hormone-binding globulin (SHBG) was measured with a non-extraction radioimmunoassay kit from Farmos Diagnostica (Oulunsalo, Finland), dehydroepiandrosterone sulphate (DHEAS) was measured with a radioimmunoassay kit from Immuno-tech (Iyon, France) and free cortisol was determined using an Amerlex radioimmunoassay kit (Amer sham International, UK).

Intra- and interassay precision ranged from 8 to 12% in our laboratories. The ACTH stimulation tests were performed with 25 µg of tetracosactide (Synthec) supplied by Ciba (Basel, Switzerland), and levels of free cortisol, 170HP and DHEAS were determined at times 0, 30, 60 and 120 min. Adrenal reserve was considered to be the value of free cortisol at 60 min (peak F), and an absolute increase was defined as the difference between the basal value of peak F and the maximum peak attained after ACTH stimulation (AbsF). The free testosterone index (FTI) was calculated with the equation: (total hormone × SGBH)/100 (16). The hormone study protocol was repeated after 3 and 9 months of treatment, and 1 month after treatment was terminated.

Blood samples for liver function tests (serum total and conjugated bilirubin concentration, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase activities) were measured every 2 months with standard methods. Patients were cautioned to discontinue treatment if nausea, abdominal pain, vomiting or jaundice appeared, and to seek medical care if any of these problems occurred. They were encouraged to telephone the authors whenever they had any questions about the treatment. All patients agreed to use barrier contraception or to abstain from intercourse during therapy. The study was designed and performed in accordance with the recommendations of the Declaration of Helsinki. The protocol was approved by our centre’s ethical committee, and all subjects gave written informed consent to participate.

Statistical analysis
We compared pretherapy measurements and values obtained after 3 and 9 months of treatment with Friedman’s test for random blocks, followed by pairwise comparisons with the Newman–Keuls method. Values obtained after 9 months of treatment and 1 month after treatment was discontinued were compared with Student’s t-test (for normal samples) or with Wilcoxon’s test. The influence of basal values on variation throughout KTZ treatment was assessed by calculating the variability index for each variable after 3 and 9 months with reference to initial values, which were assigned a value of 100. Percentage variation was correlated with basal values by calculating Pearson’s r coefficient. Correlation coefficients were found also for pairs of indices of variation after 3 and 9 months. All values were expressed as means ± SEM. A probability of p < 0.05 was taken to indicate significant differences. All statistical analyses were done with SPSS/PC+ software on an IBM PS2 personal computer.

Results
Changes in clinical symptoms
Eleven patients abandoned treatment, two for unspecified personal reasons and nine because of side-effects. Of these nine, six stopped treatment before the 3rd month and the remaining three dropped out between months 3 and 9. Only 26 women completed the 9-month course without experiencing side-effects severe enough to cause them to drop out.

The clinical symptoms of hirsutism improved in all 26 of these patients (basal score 18.3 ± 4.6 vs 12.4 ± 3.1: p < 0.001) and all patients reported subjective improvements in the texture of their hair. In the 11 patients in whom acne was associated with hirsutism, improvement was seen after 3 months (p < 0.01), with complete resolution in five patients and significant improvement in the other six. After 9 months of treatment, only one patient continued to have mild
acne. All reported a decrease in facial oil secretion and reductions in the frequency of shampooing. On comparing the number of menses during the 10 months previous to treatment and the 10-month study period, we found menstruation to be more frequent during treatment with KTZ (8.5 ± 0.6 vs 10.8 ± 0.4; p < 0.01).

Hormonal modifications

Table 1 summarizes the changes in hormone concentrations during KTZ treatment. Androgenic steroids (testosterone, FTI, androstenedione, DHEAS) (Figs. 1 and 2) decreased during the 9-month treatment period and increased after treatment was discontinued. There was a slight, non-significant increase in SHBG levels, and E₂ was increased throughout treatment, returning to basal levels when KTZ was stopped. During treatment there was a slight but non-significant tendency for basal cortisol levels to rise. No significant differences were noted in peaks F and AbsF.

Basal 17OHP levels increased throughout treatment (p < 0.001) and dropped after treatment was suspended. The levels of DHEAS decreased during treatment (p < 0.001); when KTZ was stopped, hormone levels increased but failed to recover initial levels in most patients. However, three women showed a paradoxical increase in serum DHEAS during treatment.

Between months 3 and 9, LH decreased (p < 0.05); after KTZ was suspended, this hormone decreased further (p < 0.05). No change was observed in FSH levels during or after treatment. The LH/FSH ratio decreased during months 9 and 10 (p < 0.05).

Correlation between initial hormone values and indices of variation

Initial testosterone levels were correlated inversely with the percentage of testosterone after 3 months of treatment (r = −0.65; p < 0.001). The initial FTI was correlated directly with the percentage of SHBG after 3 months (r = 0.74; p < 0.001), 9 months (0.71; p < 0.001) and 10 months (r = 0.81; p < 0.001). There was an inverse correlation between initial SHBG values and the percentage of SHBG after 3 months (r = −0.53; p < 0.01), 9 months (r = −0.67; p < 0.001) and 10 months (r = −0.65; p < 0.001).

Correlation between pairs of indices of variation

The percentage of DHEAS was correlated inversely with the percentage of SHBG after 3 months (r = −0.52; p < 0.01), 9 months (r = −0.66; p < 0.001) and 10 months (r = −0.60; p < 0.01). There was a direct correlation between the percentage of DHEAS and the percentage FTI after 3 months (r = 0.55; p < 0.01), 9 months (r = 0.57; p < 0.01) and 10 months (r = 0.54; p < 0.01). No correlation was found between the percentage of testosterone and the percentage of SHBG at any time during the study.

Side-effects

Several side-effects (Table 2) were noted, the most frequent being dyspeptic complaints and dysfunctional menstrual bleeding. One patient without psychiatric antecedents developed psychiatric symptoms suggestive of poorly characterized psychosis. No evidence of adrenal insufficiency was found. The patients contacted the authors mostly with concerns about dysfunctional menstrual bleeding.

Increased transaminase levels were found in five women, with values below 100 IU/l (range < 40 IU/l). After 9 months of KTZ treatment, AST and ALT levels in one woman were 450 and 265 IU/l, respectively; these values normalized after the drug was suspended. In all

Table 1. Evolution of hormone profiles (mean ± sem) in patients with polycystic ovary syndrome treated with ketoconazole (200 mg/12 h) during 9 months, and followed for one additional month.*

<table>
<thead>
<tr>
<th></th>
<th>Beginning N = 37</th>
<th>3rd month N = 29</th>
<th>9th month N = 24</th>
<th>10th month N = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (nmol/l)</td>
<td>2.7 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.2**</td>
<td>2.3 ± 0.3*</td>
</tr>
<tr>
<td>FTI</td>
<td>4.4 ± 0.6</td>
<td>2.8 ± 0.1</td>
<td>1.5 ± 0.4**</td>
<td>2.5 ± 0.5*</td>
</tr>
<tr>
<td>DHEAS (μmol/l)</td>
<td>8.3 ± 0.8</td>
<td>6.4 ± 0.8</td>
<td>5.1 ± 0.6**</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>26.6 ± 3.5</td>
<td>32.3 ± 3.4</td>
<td>41.4 ± 2.9</td>
<td>38.1 ± 2.4</td>
</tr>
<tr>
<td>A (nmol/l)</td>
<td>4.3 ± 0.3</td>
<td>3.3 ± 0.3**</td>
<td>2.5 ± 0.2**</td>
<td>3.0 ± 0.2*</td>
</tr>
<tr>
<td>E₂ (pmol/l)</td>
<td>139 ± 10.6</td>
<td>244 ± 34**</td>
<td>277 ± 23**</td>
<td>157 ± 11.7</td>
</tr>
<tr>
<td>F (μmol/l)</td>
<td>0.52 ± 0.03</td>
<td>0.54 ± 0.0</td>
<td>0.53 ± 0.05</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>17OHP (nmol/l)</td>
<td>4.2 ± 0.3</td>
<td>8.4 ± 0.9**</td>
<td>8.1 ± 0.9*</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>ACTH (ng/l)</td>
<td>16.3 ± 3.9</td>
<td>41.7 ± 8.1**</td>
<td>32.8 ± 6.8</td>
<td>28.0 ± 5.3</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>12.4 ± 0.6</td>
<td>13.5 ± 1.5</td>
<td>9.6 ± 1.9*</td>
<td>5.9 ± 1.0*</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>2.5 ± 0.3</td>
<td>3.3 ± 0.7</td>
<td>2.0 ± 0.3*</td>
<td>1.3 ± 0.1*</td>
</tr>
</tbody>
</table>

* T: testosterone; FTI: free testosterone index; DHEAS: dehydroepiandrosterone; SHBG: sex hormone-binding globulin; A: androstenedione; E₂: estradiol; F: free cortisol; 17OHP: 17-hydroxyprogesterone. Significant differences after 3, 9 and 10 months in comparison with initial values:*p < 0.05, **p < 0.01.
other patients, transaminase levels measured 30 days after treatment had decreased to within the normal range. No other significant alterations in liver function tests were detected, and serum calcium, phosphorus, sodium or potassium levels were not affected by the treatment.

Discussion

Studies in ovarian tissue have shown KTZ to act on 17α-hydroxylase, 17,20-lyase and 3β-OH-dehydrogenase (6). The increase in the activities of these enzymes in non-tumoral hyperandrogenic states makes this drug a potentially useful therapeutic option.

Hirsutism improved in those of our patients who completed the 9-month treatment. As found by others (8, 9), no improvement occurred in women who took KTZ for less than 6 months; the lack of improvement probably contributed to these patients' decision to stop treatment. We found no correlation between clinical improvement and hormonal modification, the most plausible explanation being the inadequacy of the Ferriman & Gallway scale, together with the heterogeneity of the clinical problems that we dealt with.

Our patients reported subjective improvements from hyperandrogenism, i.e. softening and lightening of hair and decreased scalp oil secretion. The number of dysfunctional menses was greater during treatment, a finding that may be related to the increase in E2 levels and amelioration of hyperandrogenism. However, there is no evidence that increased menses increased the frequency of ovulation. Because under this treatment the progesterone level is not a valid criterion for ovulation, it is difficult to document the occurrence of ovulatory cycles (17). Acne improved markedly within the first 3 months of treatment, and resolved completely in many cases. The relatively rapid improvement may be related to the drug's antibacterial action, in

---

**Table 2.** Side-effects of treatment with ketoconazole at a dose of 400 mg/12 h for 9 months.

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>(N =)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspeptic complaints (nausea, dyspepsia)</td>
<td>9</td>
</tr>
<tr>
<td>Dysfunctional menstral bleeding</td>
<td>8</td>
</tr>
<tr>
<td>Scalp hair loss</td>
<td>6</td>
</tr>
<tr>
<td>Urticaria</td>
<td>1</td>
</tr>
<tr>
<td>Psychiatric symptoms</td>
<td>1</td>
</tr>
<tr>
<td>Elevated transaminase levels</td>
<td>5</td>
</tr>
</tbody>
</table>
addition to modification of the hormonal environment (18).

Our patients attained a more favorable hormonal pattern, with decreased androgenic steroids of both ovarian and adrenal origin. In an earlier study, Martikainen et al. (8) found reductions only in ovarian androgenic steroids, and concluded that KTZ acts preferentially at the gonadal level. Our finding of decreased levels of DHEAS, in agreement with Venturoli et al. (9) and Akalin (12), suggests that the drug acts at both levels. During treatment, E₂ increased in our patients, a finding that is at variance with some, but not all, studies on this subject (8, 10, 12). The increase in E₂ may be related to a greater aromatization capacity.

Our data show an inverse correlation between basal SHBG values and the percentage of SHBG during KTZ treatment, which would account for the increase in SHBG levels found by Martikainen et al. (8) in a series of women with initially low levels of this protein. The decrease in androgens associated with a slight change in SHBG led to a lower, and hence more favorable, FTI. Surprisingly, there was no increase in SHBG, a change typically associated with the well-known modulatory effects of decreased testosterone and DHEAS levels and an increased E₂ level (19, 20). This finding suggests a possible direct limitation by KTZ of hepatic synthesis of SHBG.

We observed no significant alteration in basal cortisol levels, in agreement with earlier reports (2, 3, 9, 12). The response of cortisol to ACTH stimulation was not modified significantly. Together, these findings suggest that adequate gluco-corticoid reserves were maintained during KTZ treatment in these patients.

Serum levels of 17OHP increased in association with decreased testosterone and androstenedione levels, an observation that suggests that 17,20-lyase was blocked by KTZ. When treatment was discontinued, 17OHP levels decreased, approaching their initial values. This observation confirms the reversible nature of KTZ action on steroidogenesis. The levels of DHEAS dropped significantly during treatment in most of our patients; however, three women showed a paradoxical increase. We hypothesize that KTZ revealed a partial 3β-0H-dehydrogenase deficit.

The statistical analysis of basal DHEAS values and indices of variation in the present study showed that decreases in DHEAS were correlated with increased SHBG and decreased FTI. In contrast, we found no correlation between percentage modifications in SHBG and testosterone. These findings agree with the recent suggestion that adrenal androgens influence SHBG levels (20) and raise the possibility that adrenal androgen blockage by KTZ not only decreases DHEAS, a potential substrate for testosterone, but also decreases the free fraction of testosterone by interacting with SHBG.

Treatment with KTZ leads to a number of side-effects, the most frequent being nausea and dyspepsia, and dysfunctional menstrual bleeding. Although bothersome, these effects, which usually appear within the first few months, are usually not serious enough to warrant discontinuation of treatment. However, a finding of greater concern was that during the 8th or 9th month of treatment, six patients reported significant scalp hair loss, which resolved spontaneously when KTZ was suspended. Another noteworthy case was that of a single patient who developed psychiatric symptoms, suggestive of a poorly characterized psychosis. Asymptomatic increases of up to 5–10% in transaminase levels have been described in some patients in association with KTZ treatment. Potentially lethal elevations occur in approximately 1 in every 15 000 patients (21) and are more likely to appear in persons older than 50 years (22). According to Pepper et al. (10), the risk from KTZ is lower than that associated with oral contraceptives or corticoids used to treat PCOS. Although transaminases were elevated in five of our patients, none required suspension of medication; in the only patient with marked increases, AST and ALT values of 450 and 265 IU/l, respectively, did not appear until the end of the 9th month of treatment, when treatment was scheduled to be terminated. One month after discontinuation, this patient’s liver function indicators had normalized. The drop-out rate in the present study (30%) was acceptable, and was lower than the figures of attrition given for other series (8–10, 12).

Acknowledgments. This study was partially supported by a grant from the Granada University Funds of Investigation to the Unidad Metabolica Group through Project No. 193. We thank Ms Trinidad Guardia and Ms Conchi Sorroche for their competent technical assistance and Ms Karen Shashok for translating the original manuscript into English.

References


Received January 28th, 1993
Accepted December 3rd, 1993