SHORT TERM TREATMENT WITH KETOCONAZOLE: EFFECTS ON GONADAL AND ADRENAL STEROIDOGENESIS IN WOMEN

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Summary: Ketoconazole, an imidazole derivative, is a large spectrum antifungal agent. The drug is known to cause a decrease in plasma androgens and adrenal steroids in normal men; it is also an active drug in the treatment of malignant tumors of the prostate. To examine the antiandrogenic action of this drug in women, we measured several gonadal and adrenal steroids in 21 normally menstruating women before and after receiving oral ketoconazole (200 mg twice daily) for 5 days. Plasma testosterone (T) decreased from a basal level of 0.35 to 0.25 ng/ml ($\pm$ SEM) ($P<0.001$); dihydrotestosterone (DHT) from a basal level of 190.62 ± 23.2 to 159.75 ± 19.43 pg/ml ($P<0.02$); dehydroepiandrosterone sulphate (DHEA-S) from 1.42 ± 0.44 to 1.15 ± 0.19 μm/ml ($P<0.02$). Plasma 17β-estradiol (E_2) decreased from a basal level of 97.42 ± 29.37 to 54.32 ± 9.9 pg/ml ($P<0.05$). In contrast, plasma 17-OH-progesterone (17-OHP) levels increased from a basal level of 44.81 ± 8.21 to 71.81 ± 15.81 ng/100 ml ($P<0.05$). These results confirm that the ketoconazole blocks the conversion of progestins into androgens. The decrease in the plasma concentration of E_2 suggest a direct effect of the ketoconazole on the ovary. It is likely that the effect of the drug, both at the level of the ovaries and of the adrenal gland, is dose-dependent. The new therapeutic approach to hormone-dependent tumors in men using ketoconazole opens intersting therapeutic possibilities in women, such as a nonhormonal antiandrogen in certain dysfunctional pathological conditions and in the treatment of hormone-dependent tumors and hormone-secreting tumors in women.

INTRODUCTION

Among the imidazole derivatives, ketoconazole (NIZORAL; Janssen Pharmaceutica, Beerse, Belgium) is a wide spectrum antifungal agent, administered and active per os ([1]). The first report of the appearance of gynecomastia ([2]) in men under treatment with ketoconazole led to research on the action of this agent at the level of testosterone synthesis.

In 1982 Pont ([3]) demonstrated a dose-dependent inhibition of the levels of plasma testosterone (T) after the administration of 200-600 mg of ketoconazole.

Furthermore, high doses of the drug (400-600 mg) block the increase of plasma cortisol after ACTH ([4]).

As far as the mechanism of action is concerned, it has been suggested that the presence of the lateral phenylated chain of the imidazole molecule is necessary in order to cause the block of the biosynthetic pathway of T ([5]), a block which was identified by Santen ([6]) at the level of the C_{17-20} lyase.

Recent reports have shown that ketoconazole, administered in high doses, causes a marked decrease in the plasma levels of T, dihydrotestosterone (DHT) and Δ_4-androstenedione (A) to castration levels in patients suffering from advanced stage cancer of the prostate ([7]). Furthermore, positive endocrinological effects on the part of ketoconazole have been reported in a case of an ectopic adrenal tumor ([8]), and in 10 cases of Cushing's syndrome ([9]).

Finally Contreras *et al.* ([10]) reported tumor regression after treatment with ketoconazole in a patient suffering from functional metastatic adrenal carcinoma.

In gynecology, ketoconazole is normally used in the treatment of vulvovaginitis

Table 1. – *LH, FSH, 17β-estradiol (E_2), testosterone (T), dihydrotestosterone (DHT), androstenedione (A), 17-OH-progesterone (17-OHP) and dehydroepiandrosterone sulphate (DHEA-S) plasma levels in normal women during the early follicular phase, before and after 5 days of ketoconazole administration (200 mg twice daily).*

		No. cases	Basal	After ketoconazole	Significance
LH	mUI/ml	13	6.71 ± 0.78	6.24 ± 1.14	N.S.
FSH	mUI/ml	10	4.42 ± 0.67	4.83 ± 0.61	N.S.
E_2	pg/ml	11	97.42 ± 29.37	54.32 ± 9.9	$P<0.05$
T	ng/ml	20	0.35 ± 0.05	0.26 ± 0.04	$P<0.001$
DHT	pg/ml	8	190.62 ± 23.2	159.75 ± 19.43	$P<0.02$
A	pg/ml	21	2131 ± 198.4	1984 ± 222.7	N.S.
17-OHP	ng/100 ml	11	44.81 ± 8.21	71.81 ± 15.81	$P<0.05$
DHEA-S	μg/ml	20	1.42 ± 0.44	1.15 ± 0.19	$P<0.02$

caused by Candida Albicans ([1]), the most common infection of the female reproductive tract.

Since the biosynthesis of ovarian steroids in general follows the same metabolic pathways as those of the other steroid-secreting glands, it seemed to us to be of interest to chart the behaviour of the female sex steroids during a short term treatment with ketoconazole.

MATERIAL AND METHODS

The study was performed on 21 normally menstruating women, 19 to 32 years of age, after obtaining their voluntary consent. They had a history of regular menstrual cycles (mean length 29±5 days) and they had not received endocrinologically active drugs for at least 3 months before the study. All the patients, during the early follicular phase of the menstrual cycle, received oral ketoconazole (400 mg/daily) in two daily doses for 5 days; two blood samples were obtained between 8 and 9 a.m. before and 5 days after the administration of ketoconazole.

The collected heparinized samples were immediately centrifuged and plasma was deep frozen until assay. Samples of each patient were run in a single assay.

In the plasma samples T, DHT, E_2, A, dehydroepiandrosterone sulphate (DHEA-S), 17-OH-progesterone (17-OHP), LH and FSH were measured by radioimmunoassay.

Hormone determinations

T was measured by a solid phase ^{125}I radioimmunoassay (Medical System Kit) in unextracted plasma, based on testosterone-specific antibody immobilized to the wall of a polyprophylene tube.

E_2 and A plasma levels were assayed by RIA (Bio-Merieux Kit) after extraction with ether. Charcoal-dextran was used to separate bound from free fractions.

DHEA-S was measured by RIA (Bio-Merieux Kit) after dilution with a buffer causing a normal Ag-Ab reaction, and separation with charcoal-dextran, as above.

17-OHP was measured by RIA (Bio-Merieux Kit) using the same techniques as DHEA-S without the buffer solution. DHT was detected by cromatolite A column cromatography; hormone separation was performed by means of 4 ml Isoctane and 6 ml di-ethyl-acetate isoctane. LH and FSH determination was performed by commercial available kits (Sorin, Saluggia, Italy).

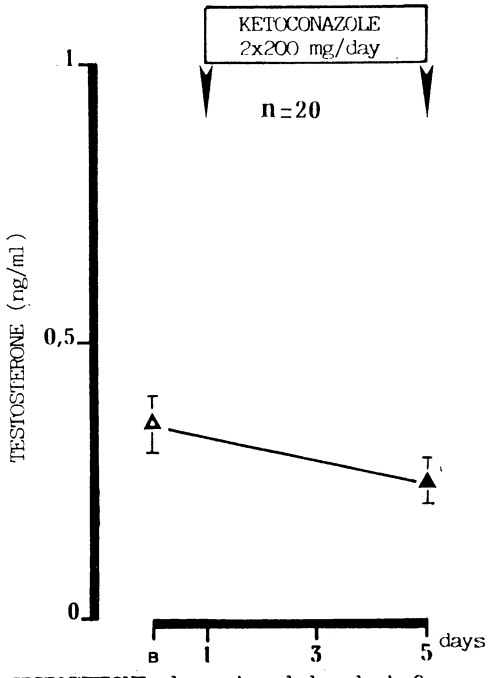
Statistical analysis of the results was performed by using the two-tailed Student's t test.

RESULTS

All results are reported as the means ± SE (table 1).

Basal plasma T levels fell from 0.35 ± 0.05 (±MSE) ng/ml to 0.26±0.04 ng/ml after the administration of the drug ($P<0.001$ vs. corresponding basal values) (fig. 1).

DHT plasma levels decreased from 190.62±23.2 (±MSE) to 159.75±19.43 pg/ml ($P<0.02$ versus corresponding basal values) (fig. 2). DHEA-S, an androgen of almost exclusively adrenal origin, presented analogous behaviour, decreasing af-



TESTOSTERONE plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

Fig. 1.

ter Ketoconazole from basal levels of 1.42±0.44 (±MSE) to 1.15±0.19 ng/ml ($P<0.02$ versus corresponding basal values) (fig. 3). Ketoconazole administration induced a significant decrease of E_2 plasma levels from 83.38±21.8 pg/ml (±MSE) to 48.66±9.9 pg/ml ($P<0.05$ versus corresponding basal values) (fig. 4).

Ketoconazole caused a significant increase of plasma basal levels of 17-OHP from 44.81±8.21 to 71.81±15.81 ng/100 ml ($P<0.05$ versus corresponding basal levels) (fig. 5).

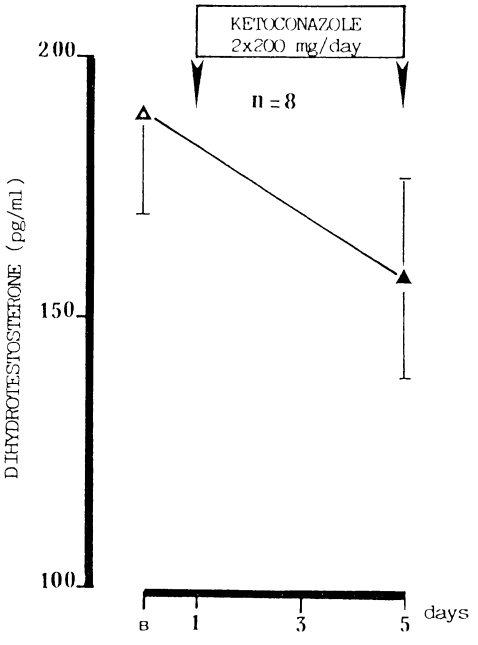
A significant decrease in the blood levels of A was not observed from basal levels of 2131±198.4 (±MSE) pg/ml to values of 1984±222.7 (±MSE) pg/ml after the ketoconazole (N.S.) (fig. 6).

DISCUSSION

Ketoconazole causes a decrease in plasma androgens and adrenal steroids in normal men ([6, 11]). Positive results have been obtained with this drug alone or in association with LHRH analogues in the treatment of malignant hormone-dependent tumors of the prostate and of the adrenal gland ([7, 10, 12, 13]). The antiandrogenic action of this antifungal drug has been confirmed by in vitro studies of testicular and adrenal tissue cultures ([14]).

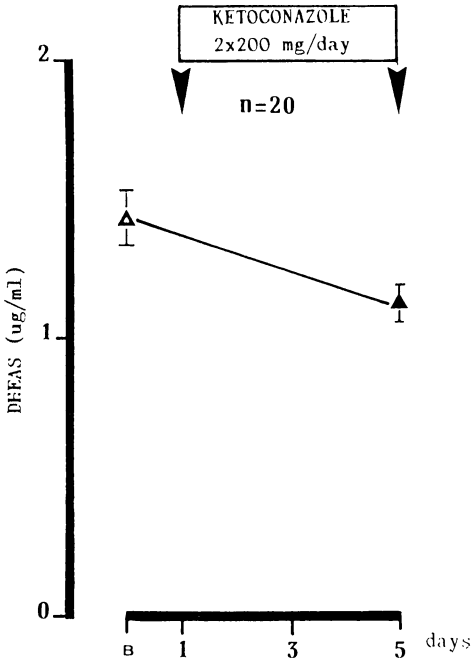
The mechanism of action of the drug seems to be linked to an interference with the enzymatic system which regulates the biosynthetic pathway of androgens, especially the Δ_4 steroid pathway ([15]).

From research conducted on tissue slices of excised human testes (from patients with prostatic cancer) it appears that ke-



DEHYDROTESTOSTERONE plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

Fig. 2.



DHEAS plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

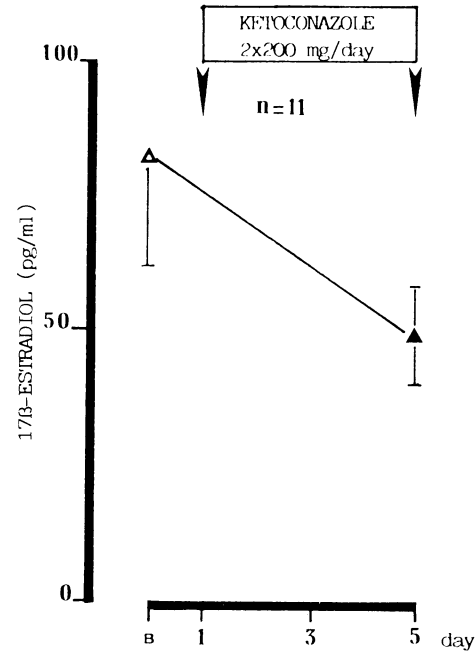
Fig. 3.

toconazole acts by inhibiting the activity of C_{17-20} lyase, 17-hydroxylase and 16-hydroxylase ([16]). This enzymatic block would cause a decrease in the quantity of T and A produced and a parallel accumulation of 17-OHP ([6]), which is the direct precursor of the two androgens.

Our data confirms the anti-androgenic activity of ketoconazole in the normal woman. The administration of ketoconazole caused a fall in the plasmatic concentration of T; a significant decrease in the E_2 was observed, in accordance with the observation of Contreras *et al.* ([10]) in a patient suffering from metastatic adrenal carcinoma, where the high levels of T and E_2 decreased dramatically after high doses of ketoconazole administered for a long period. The large reduction in the androgens was followed by an improvement in the clinical state and a decrease of the hirsutism present in the patient.

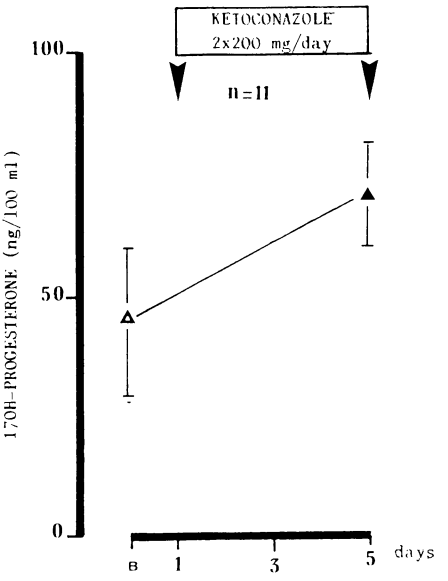
Though the sequence of reaction which leads from the C_{19} androgens to estrogens is not yet clear, we know that T and A are immediate precursors of E_2 and estrone (E_1), and therefore their secretion is accompanied by that of androgens. It is thus understandable that the decrease in T observed by us after ketoconazole was accompanied by a parallel decrease in E_2.

Whilst A, T and 17-OHP derive, in different percentages, from the adrenal gland and the gonads, we know that E_2 is produced exclusively by the ovary and that DHEA-S almost exclusively by the adrenal gland ([17]). The decrease in the pla-



17β-ESTRADIOL plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

Fig. 4.



17OH-PROGESTERONE plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

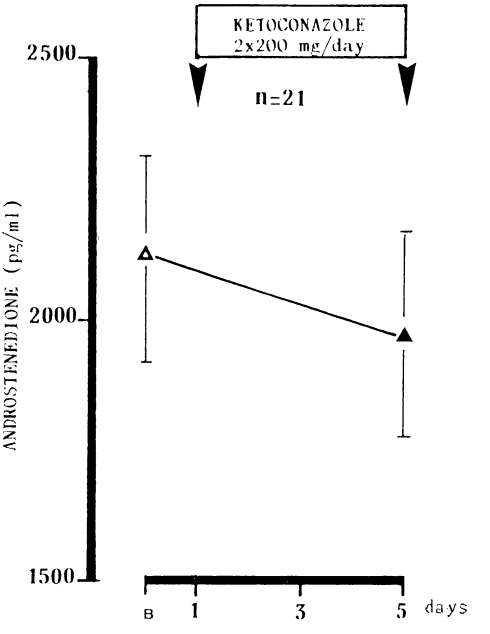
Fig. 5.

smatic concentrations of E_2 suggest a direct effect of ketoconazole on the ovary.

It would seem that the action of ketoconazole causes what normally occurs in the corpus luteum, where the preferred biosynthetic pathway is the Δ_4 steroids ([18]). The almost total lack of the desmolyase, characteristic of luteinized cells, leads to the accumulation of the progestins and their consequent low transformation in C_{19} steroids ([19]).

Our decision to use this drug in low doses and for short periods of time could explain the non significant decrease in A. In fact Williams and Bloom ([7]) obtained a marked reduction of the serum concentrations of T, DHT, A and a block of adrenal androgens in patients suffering from carcinoma of the prostate with elevated doses of the drug (1200 mg/day). It is therefore likely that the effect of the drug, both at the level of the ovaries and of the adrenal gland, is dose-dependent.

It has also been observed ([3, 6]) that in men treated with ketoconazole the decrease in T is accompanied by a compensatory increase in the plasma LH. Contradictory data have been obtained as far as regards the effect of Ketoconazole on gonadotropin secretion. Vawda and Davies ([20]) observed that a single oral dose of 24 mg of Ketoconazole/kg body weight in sexually mature rats decrease T and DHT levels without altering gonadotropin levels. Holland *et al.* ([21]) treated 3 boys 3.3 to 3.9 years old, who had precocious puberty that was unresponsive to an analogue of gonadotropin-releasing hormone, with ketoconazole for more than 12 months: LH and FSH levels showed no significant change during the first five days of therapy.



ANDROSTENEDIONE plasma basal levels before (▲) and after (▲) administration of 200 mg of Ketoconazole twice daily for 5 days.

Fig. 6.

Schurmeryer and Nieschlag ([5]) showed that after a single dose of 400 mg Ketoconazole in 5 healthy men LH, FSH and prolactin levels remained unchanged. On the other hand Santen ([6]) reported that in 10 normal men, after a single oral dose of Ketoconazole (200 mg) plasma LH increased in response to the testosterone decrease. Our results showed no change of LH and FSH plasma levels after ketoconazole administration. It has still to be proved that in women the decrease of E_2 is accompanied by modifications of the gonadotropins, expecially of FSH, so that further studies on the effects of the drug at the pituitary level, expecially in long-term treatment, are necessary.

The new therapeutic approach to hormone-dependant tumors in the male using ketoconazole ([7, 12, 22]) opens interesting therapeutic possibilities also in women. One could put forward the hypothesis that, in hormone-secreting ovarian tumors, a drug like ketoconazole, capable of causing a decrease in the steroidogenesis in the tumor, could induce a regression of the tumor. On the other hand, the inhibition of E_2 could be used to advantage in the treatment of estrogen-dependent tumors such as in endometrial and breast cancer in women.

In conclusion, ketoconazole could be of potential use for two principal indications: as a non-hormonal antiandrogen in certain dysfunctional pathological states such as hypertricosis and ovarian and/or adrenal hyperandrogenism and in the treatment of hormone-dependent tumors and hormone secreting tumors in women, either alone or in association with other drugs, as it is currently utilized in men.

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