Favourable response of a virilizing adrenocortical carcinoma to preoperative treatment with ketoconazole and postoperative chemotherapy

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Abstract. A 25-year-old woman presented with an extensive adrenocortical carcinoma with severe virilization and mild Cushing's syndrome. In the tumour there was a primacy of the P450C17 (17,20-lyase) over the P450C21 (21-hydroxylase) route, favouring the synthesis of androgens over corticoids. Preoperatively, the patient was treated with the antimycotic agent ketoconazole, a known inhibitor of steroid synthesis, at a dose of 600 mg/day and after a week 1200 mg/day, to reduce operation risks and to achieve a better metabolic control. This treatment markedly decreased hyperandrogenism and normalized the hypercortisolism. The main effect of ketoconazole was at the 17,20-lyase level and probably at a locus prior to steroidogenesis, i.e. at the P450SCC and/or 17α-hydroxylase level. In contrast with other studies no all at was seen on the 11-hydroxylase activity of P450C11. After removal of a massive adrenal carcinoma, extending into the vena cava, vena cava resection and hemihepatectomy because of liver invasion, plasma cortisol and androgen values normalized. Despite adjuvant chemotherapy with o,p'-dichlor-diphenyl-dichloretan (4000 mg daily) hyperandrogenism soon recurred and lung metastases became manifest. Within 2 months after starting combined chemotherapy with 5-fluorouracil, cisplatin, and doxorubicin lung metastases almost completely disappeared with clinical and biochemical resolution of the hyperandrogenic state.

The prognosis of adrenocortical carcinoma is very poor (1). Temporary objective improvement in patients with inoperable or recurrent adrenocortical carcinoma has been achieved by o,p'-dichlor-diphenyl-dichloretan (DDD) therapy (2,3). Very recently the use of ketoconazole has been suggested in the preoperative management of adrenocortical carcinoma. Ketoconazole is a broad spectrum antimycotic agent which is a potent inhibitor of enzymes of the cytochrome P450 system in gonadal but also in adrenal steroid synthesis (4,5). It has been used preoperatively in Cushing's syndrome with virilization due to adrenocortical carcinoma to reduce the increased operation risk of a higher bleeding tendency, poor wound healing and thromboembolism associated with hypercortisolism, and to achieve a better metabolic control (6-8).

We present a patient with metastasized adrenocortical carcinoma with severe virilization and mild Cushing's syndrome in whom preoperative therapy with the enzyme inhibitor ketoconazole resulted in short-term clinical improvement of both cortisol and testosterone hypersecretion. Hyper-tesosteronemia and intrapulmonary metastases, occurring postoperatively despite o,p'-DDD adjuvant therapy, were successfully treated by combined chemotherapy with 5-fluorouracil, cisplatin and doxorubicin.

Patient and Methods

Case report
A 25-year-old woman was hospitalized because of virilization. In the last nine months, starting in December 1988, there was a weight gain of 20 kilogram with round-
ing of the face, fullness of the cheeks, increased fat deposition around the girdle, broadening of the shoulders, development of male musculature and deepening of the voice. March 1989 excessive hair growth appeared on her chin, upper lip, arms and legs. A month later a progressive acne developed. June 1989 she stopped the use of oral contraceptives and amenorrhea occurred.

On physical examination the salient features included virilization with male musculature, broad shoulders and deep voice. Blood pressure was 130/90 mmHg. Excessive coarse hair growth was present on the upper lip, chin, the site of whiskers, chest, upper pubic triangle, arms and legs. The skin showed extensive acne and striae. The patient had a moon face and supraclavicular fat pads. The clitoris was enlarged by 3 cm. In the abdomen a right-sided mass with a diameter of approximately 15 cm was palpable.

Routine hemato logic and biochemical examinations were unremarkable. Endocrine studies showed dramatically increased plasma levels of testosterone (T) (64.4 nmol/l, normal value in women <2.4 nmol/l), androstenedione (A4) (100.5 nmol/l, normal value <9.6 nmol/l), 17 OH-progesterone (17 OHP) (165 nmol/l, normal value <8.8 nmol/l), and dehydroepiandrosterone sulphone (DHEA-S) (33.0 µmol/l, normal value <9.9 µmol/l). Plasma estradiol (E2) and dehydroepiandrosterone (DHEA) levels were not elevated. Morning plasma cortisol was increased (0.70 µmol/l, normal value <0.55 µmol/l) with a lack of diurnal rhythm; plasma 11-deoxycortisol was strongly elevated (61.3 nmol/l, normal value <6.1 nmol/l). There was a high urinary 24-h excretion of 17-ketosteroids (274 µmol) and of 17-hydroxycorticoids (262 µmol) (normal value <52 µmol in both cases).

Increasing doses of dexamethasone up to 16 mg daily did not suppress cortisol or androgen production. Stimulation with 0.25 mg ACTH(1-24) iv or 100 µg ovine CRH iv did not result in an increase in cortisol or androgen levels.

Abdominal ultrasonography, computerized tomography and magnetic resonance imaging showed a mass of 10.5 x 7.5 x 15 cm, in the right abdominal region. The margins of the inferior vena cava adjoining the tumour were not clearly demarcated and there was almost certainly invasion of the liver by the tumour. Angiography and venography revealed an extremely narrowed inferior vena cava with compression and probably invasion. On chest films there were no signs of intrapulmonary metastases.

In conclusion, a virilizing carcinoma with Cushing's syndrome was suspected on the basis of a large adrenal mass, rapidly progressive signs and symptoms, and biochemical evidence of increasing levels of androgens and corticoids.

In preparation for surgery the patient was given ketoconazole, the first week 200 mg and the second week 400 mg tid. Three weeks after starting ketoconazole therapy (September 14, 1989) she underwent surgery (Prof. Dr MJH Slooff, University Hospital Groningen, The Netherlands). A tumour mass with a size of 15 x 14 x 9 cm, originating from the right adrenal gland was resected. Because of invasion of the liver by the tumour a right hemihepatectomy with cholecystectomy was performed. The tumour also extended through the right adrenal vein into the inferior vena cava; a thrombus together with a part of the vena cava was resected and the defect was closed with a vena cava donorparch.

The histologic findings included pleomorphic cells, pleomorphic and hyperchromatic nuclei, and a variable mitotic activity. There was invasion of the capsule and into the liver; the section margins of the liver were free of tumour. The tumour extended through the right adrenal vein into the inferior vena cava. There were no signs of metastases in regional lymph nodes or in the gallbladder.

Postoperatively (October 11, 1989) treatment with o,p'-DDD was started at a dose of 4000 mg/day supplemented with cortisol acetate 12.5 mg bid and 9-fluorohydrocortisone (Florinef® 0.1 mg/daily), when signs and symptoms of adrenocortical insufficiency appeared. However, soon plasma testosterone levels, which had been normalized after surgery, again increased to a peak value of 23 nmol/l and four months after starting o,p'-DDD intrapulmonary metastases became manifest. Combined chemotherapy with 5-fluorouracil (500 mg/m²/day at day 1 to 3, cisplatin 120 mg/m², and doxorubicin 60 mg/m² both at day 2 was started (February 14, 1990) at monthly intervals and continued for 6 months. Within a month intrapulmonary metastases dramatically improved and later almost completely resolved with clinical and biochemical resolution of the patients hyperandrogenic state. Unfortunately, 6 months after starting chemotherapy a serious polyneuropathy developed, probably due to cisplatin neurotoxicity.

The plasma levels of 17 OHP, A4, T, E2 and DHEA were measured by RIA after diethylether extraction and paper chromatography in a modified Bush A system (9-11). Plasma 11-deoxycortisol was measured by RIA after dichloromethane extraction and paper chromatography in a Bush B3 system. The anti-11-deoxycortisol serum had been raised in sheep against an 11-deoxycortisol-21-hemisuccinate-BSA conjugate. The inter-assay coefficient of variation (CV) was 9.3% at a level of 1.39 nmol/l (N=10). The intra-assay CV expressed as the duplicate variation was 5.2% in a range of concentrations from 0.88 to 5.1 nmol/l (N=25). Radioimmunoassay of plasma cortisol was performed following heat denaturation of plasma binding proteins (12) Plasma DHEA-S was measured by direct RIA. The inter-assay CV was 5.6% at a level of 5.85 µmol/l (N=8); the intra-assay CV at this level was 4.6% (N=10). Urinary 17-ketosteroids and 17-hydroxycorticoids were measured according to the method of Appleby et al. (13).

Statistical analysis was performed by testing the means of Spearman's rank correlation coefficients.
Results

Ketoconazole at a dose of 200 and later 400 mg significantly decreased plasma 17 OHP, cortisol, A₄, T, E₂, DHEA and DHEA-S (Figs. 1 and 2). Plasma 11-deoxycortisol remained virtually unchanged. A significant correlation was found between the ketoconazole induced changes in plasma level of 11-deoxycortisol and, respectively, 17 OHP, cortisol, A₄, T, E₂, DHEA and DHEA-S (R=0.76-0.93, p<0.001-0.01). It has to be stressed that during ketoconazole treatment the plasma 17 OHP and 11-deoxycortisol levels remained many-fold increased in contrast to normalization of cortisol, A₄ and DHEA. Plasma T showed a dramatic decrease but did not normalize.

Considering the ratios of 17 OHP over 11-deoxycortisol and 11-deoxycortisol over cortisol, there was no significant effect of ketoconazole treatment (Fig. 1). The ratio of 17 OHP over A₄, however, showed a 5-fold increase, whereas the A₄ over T ratio slightly though significantly decreased during ketoconazole therapy (Fig. 2).

After surgery plasma, 17 OHP (4.9 nmol/l), A₄ (4.2 nmol/l), DHEA (4.2 nmol/l) and T (1.1 nmol/l) were completely normal, but despite o,p'-DDD therapy gradually increased to maximum levels of, respectively, 98 (17 OHP), 51 (A₄), 14 (DHEA), and 23 (T) nmol/l. Within 2 months after starting combined chemotherapy all values had returned to normal (4.2, 1.8, 3.3 and 1.6 nmol/l), respectively. Six months later plasma testosterone levels were still in the (low) normal range (0.5 nmol/l).

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**Fig. 2.**
The effect of ketoconazole (first week 200 mg thrice daily, second week 400 mg thrice daily) on the adrenal androgen biosynthesis in a patient with adrenal carcinoma, virilization and Cushing's syndrome. The horizontal line indicates the upper level of normal, the vertical lines the onset of ketoconazole treatment, respectively, 600 mg and 1200 mg daily. The p-values point to the whole ketoconazole treatment period. Note the normalization of cortisol despite lack of any effect on 11-hydroxylase activity (S/F ratio) or 21-hydroxylase activity (17 OHP/S ratio). 17-OHP: 17 OH-progesterone; S: 11-deoxycortisol; F: cortisol.

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**Fig. 1.**
The effect of ketoconazole (first week 200 mg thrice daily, second week 400 mg thrice daily) on the cortisol biosynthesis in a patient with adrenal carcinoma, virilization and Cushing's syndrome. The horizontal line indicates the upper level of normal, the vertical lines the onset of ketoconazole treatment, respectively, 600 mg and 1200 mg daily. The p-values point to the whole ketoconazole treatment period. Note the normalization of cortisol despite lack of any effect on 11-hydroxylase activity (S/F ratio) or 21-hydroxylase activity (17 OHP/S ratio). 17-OHP: 17 OH-progesterone; S: 11-deoxycortisol; F: cortisol.
Discussion

Adrenal carcinoma presenting with serious virilization and concurrent Cushing’s syndrome is rare. The anabolic effects of the extremely (30-fold) elevated androgens which counteracted the catabolic actions of the mildly increased (1.5-fold) glucocorticoids may be responsible for the increase in body musculature and the lack of muscular weakness and truncal obesity in the propositus.

The dramatically increased plasma androgen and 17 OHP levels in association with only mild hypercortisolism suggest that unlike in the normal adrenal cortex, in the tumour there was a primacy of the P_{450}C_{17} (17,20-lyase) over the P_{450}C_{21} (21-hydroxylase) route, favouring the synthesis of 17-OHP, DHEA, A_4 and T over cortisol. An increase in P_{450} reductase over P_{450}C_{17} in the tumour and thereby increased electron availability may be an important factor determining whether a steroid will undergo 17,20-bond scission after 17α-hydroxylation rather than 21-hydroxylation (14). Alternatively, relative deficiency of P_{450}C_{21}, as evidenced by the increased 17-OHP/11-deoxycortisol ratio, and even more relative deficiency of P_{450}C_{11}, as evidenced by the high 11-deoxycortisol/cortisol ratio, may account for the hypercortisolism being only mild in this patient (7,8).

The antimycotic drug ketoconazole reportedly blocks the mitochondrial P_{450} enzymes P_{450}SCC (cholesterol side-chain cleavage enzyme), P_{450}C_{11} (11-hydroxylase, 18-hydroxylase and 18-methyl-oxidase), and P_{450}C_{17} (17α-hydroxylase and 17,20-lyase) in the adrenal, testis and ovary. The efficacy of ketoconazole to inhibit cortisol production led to its trial as an inhibitor of steriodogenesis in cases of cortisol overproduction (15-17). Very recently its efficacy was also demonstrated in patients with virilizing and feminizing adrenal carcinoma with or without hypercortisolism (6,7,18,19).

In our patient, ketoconazole at a dose of 200 mg, later 400 mg, thrice daily, dramatically lowered plasma A_4 and T levels in the presence of still high 17 OHP levels. Consequently the 17 OHP over A_4 ratio manyfold increased, reflecting marked inhibition of P_{450}C_{17} enzyme activity (17,20-lyase). However, in addition to 17,20-lyase inhibition, persistence of the close correlation between 17 OHP and A_4 during ketoconazole therapy (R=0.96), also points to suppression of steroid synthesis at a locus prior to 17 OHP i.e. at the 17α-hydroxylase or P_{450}SCC. The ratios of 17 OHP over cortisol, 17 OHP over 11-deoxycortisol and 11-deoxycortisol over cortisol remained unchanged during the treatment period, indicating lack of any measurable effect of ketoconazole on P_{450}C_{21} and P_{450}C_{11} enzyme activities. In the absence of a measurable change in the 17 OHP over cortisol ratio, the transient decrease in plasma cortisol levels therefore could be simply explained by the relatively decreased availability of 17 OHP substrate. Nevertheless, ketoconazole given preoperatively to achieve better metabolic control and to reduce the risks of operation markedly decreased the hyperandrogenism and the hypercortisolism in our patient with virilizing adrenal carcinoma and Cushing’s syndrome.

After surgery hyperandrogenism soon returned despite o.p'-DDD adjuvant treatment, and lung metastases became manifest 4 months after operation. Combined chemotherapy with 5-fluorouracil, cisplatin and doxorubicin as proposed by Schlumberger et al. (20) was started, eliciting a dramatic response on adrenal androgen hypersecretion and almost complete resolution of lung metastases within 1 to 2 months. Serious neurotoxicity, however, occurred. In the study by Schlumberger et al., a complete remission was observed in 1 out of 5 patients with adrenocortical carcinoma and a partial response in 2. Toxicity was mild (cardiotoxicity), no neurotoxicity was reported.

Summarizing the present study illustrates the beneficial effect of the antimycotic agent ketoconazole on hypercortisolism and hyperandrogenism in the preoperative management of adrenocortical carcinoma. It further demonstrates that chemotherapy with 5-fluorouracil, cisplatin, and doxorubicin may be an active combination for the treatment of metastatic adrenocortical carcinoma.

Addendum

Ten months after starting combined chemotherapy plasma testosterone levels again increased, necessitating changing chemotherapy January 1991.

References


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