Effects of high-dose ketoconazole treatment on adrenal mineralocorticoid biosynthesis in dogs and rats

R. De Coster, M. C. Coene, C. Haelterman, D. Beerens and N. Goeminne

Laboratory of Endocrinology, Department of Life Sciences, Janssen Research Laboratories, Belgium

Abstract. At high doses, ketoconazole blocks both testicular and adrenal androgen biosyntheses and partially inhibits the glucocorticoid production. To investigate the effects of this imidazole derivative on the mineralocorticoid biosynthesis, 7 male mongrel dogs received a single oral dose of 15 mg/kg of ketoconazole or placebo, in a cross-over way. From 2 to 4 h after treatment, an iv infusion of angiotensin II (10 ng/kg per min) was performed. Ketoconazole treatment significantly blunted the aldosterone and cortisol increment, whereas 18-hydroxycorticosterone, corticosterone, 11-deoxycorticosterone (DOC), progesterone, and 17α-hydroxyprogesterone rose to peak concentrations, respectively 2.5-, 6-, 8-, 2.5- and 1.5-fold higher than those observed after placebo administration. Plasma 11-deoxycorticisol and renin activity levels remained similar in both groups. On the other hand, 2 × 2 groups of 10 male adult rats each were fed with a normal or a sodium-depleted diet. Of the two sets of groups, one was treated ip with ketoconazole (20 mg/kg twice a day), the other with vehicle solution. In animals on either diet, ketoconazole lowered 18-hydroxycorticosterone and aldosterone concentrations. Plasma DOC rose up to 25-fold in the salt-deprived animals. Serum Na⁺, Cl⁻, corticosterone and plasma renin activity remained unaffected by the treatment. These results show that high-dose ketoconazole treatment partially inhibits the biosynthesis of aldosterone by affecting the cytochrome P-450_18β. However, this inhibition was detected mainly after angiotensin II or salt-depleted stimulation of the aldosterone production. Also, the renin increase was not modified. Therefore, the accumulation of precursors with mineralocorticoid activity such as DOC might have contributed to the maintenance of a normal ionic homeostasis.

At high doses, ketoconazole, an imidazole antifungal drug, blocks both testicular and adrenal androgen biosyntheses, mainly by inhibiting the cytochrome P-450_17α/17,20-lyase (Bhasin et al. 1986; De Coster et al. 1986a; Heyns et al. 1985; Santen et al. 1983; Vanden Bossche et al. 1985, 1986). Therefore, this drug, or some derivatives may prove useful in the treatment of androgen-dependent diseases such as prostate cancer (Trachtenberg 1984; Amery et al. 1986).

During high-dose therapy for prostatic cancer (400 mg three times a day), the basal plasma cortisol and aldosterone levels are not significantly modified, but the cortisol response to ACTH-challenges is blunted (Pont et al. 1984; Heyns et al. 1985; De Coster et al. 1986a, 1987). The rise in 11-deoxycortisol, 11-deoxycorticosterone (DOC), and corticosterone observed in such patients (De Coster et al. 1986a, 1987) is in keeping with a partial inhibition of the cytochrome P-450_18β as reported in vitro (Loose et al. 1983; Vanden Bossche et al. 1986). In most cases, a compensatory increase in ACTH seems capable of overcoming the inhibition of cortisol production. Nevertheless, symptoms suggestive of hypoadrenalism have been reported (for review see Amery et al. 1986) and a systematic replacement corticosteroid therapy may be recommended. The cytochrome P-450_18β also catalyzes the various steps in the biosynthetic metabolism of aldosterone, starting from 11-deoxycorticosterone (Okamoto et al. 1985; Yanagibashi et al. 1986). In fact, an inhib
tion on the 18-hydroxylaition of corticosterone and of its conversion to aldosterone has been shown in bovine adrenal mitochondria (Nagai et al. 1986). However, the information on the effects of ketoconazole on the renin-angiotensin-aldosterone system in vivo is poorly documented. In prostatic cancer patients, receiving ketoconazole high-dose therapy, basal plasma aldosterone levels were not modified, whereas its precursors rose markedly (De Coster et al. 1986a, 1987). We studied the plasma mineralocorticoid hormones, the renin activity, and the main ion levels after stimulation by angiotensin II or by salt-deprivation, in dogs and rats respectively.

**Materials and Methods**

**Angiotensin II-stimulated aldosterone production in dogs**

Seven adult male labradors weighing 25–32 kg were used in this study. They were housed in individual rooms and maintained on dry chow ad libitum. Food, but not water, was withdrawn on the evening before the experimental day. Between 08.00 and 09.00 h, a catheter was inserted into the foreleg vein. In a randomized cross-over way, each dog received orally either a dose of 15 mg/kg of ketoconazole (suspension at 80 g/l) or a placebo. The interval between the two treatments was at least 2 weeks. From 2 to 4 h after drug administration, an iv injection of angiotensin II was given [10 ng/kg per min, diluted in gentran 40, 10%, (Travenol, Lessiner, Belgium) containing 5% glucose] at a rate of 0.2 ml/min.

Blood was collected on EDTA (0.03 mol/l) via the catheter, in chilled tubes, before treatment and 120, 150, 180, 210, 240 and 270 min thereafter. It was immediately centrifuged at 4°C and plasma was stored at −20°C.

**Aldosterone production in rats on normal or sodium-depleted diet**

Forty male Wistar rats, three months old, were kept under constant conditions and divided into 2 comparable groups. All received ad libitum a sodium-restricted diet (C1036, Altromin, Lage, Germany) containing 65 mg of NaCl/100 g. Twenty animals received distilled water, whereas the other 20 received distilled water containing 1% NaCl. After 1 month, 10 rats of each group received ip 20 mg/kg of ketoconazole diluted in HCl 0.1 mol/l, twice a day. Ten control rats were similarly treated with the same amount of vehicle solution (0.1 ml/100 g body weight). On day 12 of the treatment, 5 h after ketoconazole administration, the animals were sacrificed by decapitation to avoid the stress of ether anaesthesia. Blood was handled as described before. Another fraction of blood from each rat was allowed to clot and serum was used for testosterone, corticosterone and ion determination.

**Hormones and ions determinations**

Plasma or serum testosterone, cortisol, 11-deoxycortisol, aldosterone, 11-deoxycorticosterone, progesterone, and 17α-hydroxyprogesterone were determined as described before (De Coster et al. 1984, 1986a).

Corticosterone was assayed after extraction and high-pressure liquid chromatography (HPLC) separation in dog plasma, and measured directly, after appropriate dilution in rat serum and heating at 98°C for 10 min. Plasma 18-hydroxycorticosterone was measured by a similar radioimmunoassay, after separation by HPLC (Schönhoffer et al. 1981), using an antiserum kindly provided by Dr Bahr. Plasma renin activity levels (generated angiotensin I · ml⁻¹ · h⁻¹ at 37°C) were assayed using a commercial kit with iodinated tracer and coated antibody (Medgenix, Fleurur, Belgium). Sodium, potassium, chloride, calcium and inorganic phosphate were measured in serum with an Acuchem Microanalyzer (Ortho, Rariton, NJ, USA).

**Statistical analysis**

Student’s paired t-tests (two-tailed) were employed to compare values during placebo or drug administration at each time point for the dog study. For the rat study, statistical analysis was performed by two-way analysis of variance. In case of a significant interaction term, Student’s t-test was used to evaluate the effect of ketoconazole treatment in each of the diet groups. No statistical analysis was performed on the data on DOC and 18-hydroxycorticosterone in rats, because these determinations had to be done on pooled samples (N = 5).

**Results**

**Angiotensin II-stimulated aldosterone production in dogs**

The plasma levels of renin activity, aldosterone, cortisol, and their precursors are shown in Fig. 1. Ketoconazole treatment markedly blunted the 2-, 2.5-fold rise of plasma aldosterone and cortisol levels observed in control animals, whereas plasma renin activity was not modified. In ketoconazole treated animals, 18-hydroxycorticosterone, corticosterone, DOC, progesterone, and 17α-hydroxyprogesterone rose to peak concentrations, respectively 2.5-, 6-, 8-, 2.5- and 1.5-fold higher than those measured after placebo administration. 11-
Effects of a single oral ketoconazole administration (15 mg/kg) on the main plasma adrenal steroids concentrations and plasma renin activity (PRA) in 7 labradors. The results are expressed as mean ± SEM (* P ≤ 0.05). (---: ketoconazole; ----: placebo).

Fig. 1.
Deoxycortisol was almost identical in the two groups.

Aldosterone production in rats on normal or sodium-depleted diet

The salt-depleted diet significantly reduced the rat body weight and increased the relative weights of testes and seminal vesicles ($P \geq 0.05$, Fig. 2), whereas no significant difference could be detected for the prostate weight. In salt-deprived animals, serum $\text{Na}^+$ was lowered ($P = 0.0001$) and serum $\text{PO}_4^{2-}$ was increased ($P \geq 0.005$). Serum $\text{Ca}^{2+}$ and $\text{Cl}^-$ concentrations were not affected by the diet (Fig. 3). Potassium levels were not modified by diet or treatment, but were higher than normal values, owing to the method of blood sampling.

As expected, plasma aldosterone and renin ac-

![Graph](normal_diet.png)

**Normal diet**

- **Prostate**
  - **Control**
  - **Ketoconazole**

![Graph](salt-depleted_diet.png)

**Salt-depleted diet**

- **Prostate**
  - **Control**
  - **Ketoconazole**

**Fig. 2.**

Effects of ketoconazole (20 mg/kg twice a day, ip) on body weight, and relative weight of testis, prostate and seminal vesicle in male rats receiving a normal or a sodium-depleted diet. The results are expressed as mean ± SEM, $N = 10$ ($^* P \leq 0.05$).
tivity levels markedly rose in the animals receiving the sodium-depleted diet ($P = 0.001$, Fig. 4). Serum corticosterone and testosterone were not modified by the diet (Fig. 4). Ketoconazole treatment lowered the body weight in the rats on the salt-depleted diet ($P = 0.02$) and the relative testis weight in the other group of animals ($P = 0.04$). Prostate weight slightly decreased after treatment ($P = 0.03$), but the difference did not reach the level of significance in either group if studied separately (Fig. 2).

Ketoconazole treatment slightly lowered $\text{Ca}^{2+}$ ($P = 0.04$) and increased inorganic phosphate ($P = 0.01$, Fig. 3) in the animals on normal diet, but these variations were too small to be of biological relevance.

Serum testosterone concentrations were markedly lowered by the treatment in both groups of

---

**Fig. 3.** Effects of ketoconazole (20 mg/kg twice a day, ip) on serum $\text{Na}^+$, $\text{Ca}^{2+}$, $\text{Cl}^-$ and inorganic phosphate in male rats receiving a normal or a sodium-depleted diet. The results are expressed as mean ± SEM, $N = 10$ ($^* P \leq 0.05$, $^{**} P \leq 0.001$).
Ketoconazole treatment also almost completely prevented the rise of aldosterone in the salt-depleted animals ($P = 0.0001$) and slightly inhibited the aldosterone production in the animals on the normal diet ($P = 0.007$), but plasma renin activity remained similar in the two groups (Fig. 4). A slight, but not significant increase in plasma renin activity was observed in the animals receiving the salt-deprived diet and treated by ketoconazole. In the animals receiving the normal diet, plasma DOC levels rose from $3.5 \pm 0.6$ to $100 \pm 16$ nmol/l, whereas plasma 18-hydroxycorticosterone fell from $3.6 \pm 1.3$ to $2.8 \pm 0.8$ nmol/l. This latter effect was even more pronounced in the animals on the sodium-depleted diet, in which DOC rose from $5.5 \pm 3.6$ to $136 \pm 29$ nmol/l and 18-hydroxycorticosterone fell from $34.6 \pm 5.6$ to $13.3 \pm 6.0$ nmol/l. Serum corticosterone concentrations were not affected at all by the treatment (Fig. 4). Eight rats out of 10 on the normal diet and treated by ketoconazole showed diarrhoea and scrotal oedema from day 8 to 12 of treatment. No other adverse reaction was noted.

*Fig. 4.* Effects of ketoconazole (20 mg/kg twice a day, ip) on serum testosterone, corticosterone, plasma renin activity, and aldosterone levels in male rats receiving a normal or a sodium-depleted diet. The results are expressed as mean ± SEM, $N = 10$ (*$P \leq 0.05$, **$P \leq 0.001$).
Discussion

The ketoconazole-induced blunting of the aldosterone response to angiotensin II and to salt-depleted diet, together with a rise of DOC and, to a lesser extent, of corticosterone in dogs is consistent with a partial inhibition of the cytochrome P-450$_{18}$ (Loose et al. 1983; Nagai et al. 1986; Vanden Bossche et al. 1986). In purified bovine mitochondrial P-450$_{18}$, a striking contrast was found between the inhibition by ketoconazole of the conversion of DOC to corticosterone (IC$_{50}$: 1.3·10$^{-6}$ mol/l) and of corticosterone to 18-hydroxycorticosterone (IC$_{50}$: 0.06·10$^{-6}$ mol/l) (Nagai et al. 1986). No data are available as yet for the last step of aldosterone biosynthesis. The rise in 18-hydroxycorticosterone in dogs might be related to a failure to convert that steroid into aldosterone or to an increased secretion by the zona fasciculata as described in human 17α-hydroxylation deficiency (Kater et al. 1982). In rats on sodium-depleted diet, both aldosterone and 18-hydroxycorticosterone fell, without modification of the corticosterone levels. The decrease of 18-hydroxycorticosterone in this case might be related to an inhibition of the 18-hydroxylation of corticosterone or might be secondary to elevated DOC levels. These rather controversial data stress the fact that dose and species differences are important. This has been already demonstrated for the conversion of 11-deoxycorticisol to cortisol, which is much more sensitive to ketoconazole in the rat and bovine (Pont et al. 1982; Vanden Bossche et al. 1986) than in the guinea pig adrenal (Lambert et al. 1986).

In spite of a marked fall of the aldosterone concentration, plasma renin activity levels were not significantly modified by the treatment, either in dogs or in rats on both diets. This is probably due to the accumulation of precursors with mineralocorticoid activity, such as DOC, which inhibit renin secretion and guarantee the maintenance of a normal ionic homeostasis. On the other hand, the increment of DOC, in the presence of normal basal aldosterone levels in the rats on the normal diet receiving ketoconazole might be related to the diarrhoea and scrotal oedema.

The slight increase in plasma cortisol observed in the controls might result from a stimulating effect of angiotensin on the pituitary-adrenal axis (Ramsay et al. 1978) or from a direct steroidogenic effect on the zona fasciculata. Ketoconazole treatment blocks this response, and this inhibition is similar to its blunting effect on the cortisol response to ACTH challenge reported in man (Pont et al. 1984; De Coster et al. 1987).

In contrast, basal plasma testosterone fell in all the treated animals, suggesting that the androgen biosynthesis is, at least in vivo, more sensitive to ketoconazole than the gluco- and mineralocorticoid biosyntheses. The rise of 17α-hydroxyprogesterone and of progesterone reported in the dog study is most probably due to the blockade by ketoconazole of cytochrome P-450$_{17α,17,20-lyase}$ (Bhasin et al. 1986; Santen et al. 1983; Vanden Bossche et al. 1984, 1986; Kan et al. 1985; Rajfer et al. 1986; De Coster et al. 1986a).

The cytochrome P-450$_{17α,17,20-lyase}$ has been shown to catalyse both the 17-hydroxylase and the 17,20-lyase steps in the porcine and guinea pig adrenal and in the pig testis (Kominami et al. 1982; Nakajin et al. 1984). However, several reports demonstrated in vitro that ketoconazole differently inhibits the 17-hydroxylase and the 17,20-lyase activities in rat testicular microsomes (Rajfer et al. 1985; Vanden Bossche et al. 1985), in dispersed mouse testicular cells (Lambert et al. 1986), and in human testis (Rajfer et al. 1986). No effect of ketoconazole on the cytochrome P-450$_{11b}$ has been observed so far (Miksch & Engelhardt 1985; Vanden Bossche et al. 1986). Therefore, the rise of 17α-hydroxyprogesterone in dogs, after angiotensin- or LHRH-stimulation (De Coster et al. 1986b) is likely to be due to a blockade of the 17,20-lyase activity of the cytochrome P-450$_{17α/17,20-lyase}$.

The results reported here, together with already published data on the endocrine profile of ketoconazole high dose therapy in man (Pont et al. 1984; Heyns et al. 1985; De Coster et al. 1986a, 1987) show that this treatment mainly blocks the testicular and adrenal androgen production and partially inhibits the adrenal cytochrome P-450$_{11b}$. In a few patients, the interference of ketoconazole with the gluco- and mineralocorticoid pathways has been related to some impairment of the adrenal function (for review see Amery et al. 1986). In addition, the accumulation of DOC may be related to the occurrence of peripheral oedema and hypertension as reported in a few patients (Baert, 1985; Aabo, Amery, personal communications). Therefore substitutive corticoid therapy is recommended in patients treated with high-dose ketoconazole.
Acknowledgments

The authors are grateful to Dr. H. Vanden Bossche and Dr. W. Amery for stimulating comments, to Prof. Dr. Oelkers and Dr. Bahr (Med. Klinik Schwerpunkt, Berlin) for the 18-hydroxycorticosterone antibody, to J. Peeters for ion determination, to J. d'Aubisou and W. Van Gerven for skilful assistance in the dog experiments, to L. Wouters for statistical analysis, to L. Leijssen and to D. Verkuringen for preparing the manuscript. This work was partly supported by a grant from I.W.O.N.L.

References


Received November 7th, 1986.
Accepted March 30th, 1987.

Dr R. De Coster,
Laboratory of Endocrinology,
Department of Life Sciences,
Janssen Pharmaceutica,
B-2340 Beerse,
Belgium.