Ketoconazole Blocks Cortisol Secretion in Man by Inhibition of Adrenal 11β-Hydroxylase

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Summary. We investigated basal and ACTH stimulated levels of cortisol, corticosterone, 17α-hydroxyprogesterone, 11-deoxycortisol and 11-deoxy cortisol as well as plasma levels of ACTH before and during the oral administration of ketoconazole in five patients with Cushing's syndrome (3 with bilateral adrenal hyperplasia, 1 with adrenal adenoma and 1 with adrenal carcinoma) and in three controls. The influence of ketoconazole on the transformation of 3H-17α-hydroxyprogesterone to 3H-11-deoxycortisol and 3H-cortisol and of 3H-11-deoxycortisol to 3H-cortisol as well as of 3H-11-deoxycorticosterone to 3H-corticosterone was also examined in slices or homogenates of normal and hyperplastic adrenal tissue from four patients. Ketoconazole induced a rise of 11-deoxycortisol and 11-deoxycorticosterone, but not of cortisol and inconsistantly of corticosterone which were increased by ACTH. Thus the ratio 11-deoxycortisol/cortisol rose more after ketoconazole than after ACTH and the ratio 11-deoxycorticosterone/corticosterone rose after ketoconazole but fell after ACTH. Plasma ACTH levels were stimulated 2–50 fold by ketoconazole. Incubation studies of adrenal tissue slices with 3H-17α-hydroxyprogesterone showed that ketoconazole inhibited the transformation of 3H-17α-hydroxyprogesterone to 3H-cortisol but not to 3H-11-deoxycortisol so that the ratio 3H-11-deoxycortisol/3H-cortisol increased 15–80 fold. After incubation of adrenal slices with 3H-11-deoxycortisol or 3H-11-deoxycorticosterone and ketoconazole, a 2–260 fold increase of the ratios 3H-11-deoxycortisol/3H-cortisol and 3H-11-deoxycorticosterone/3H-corticosterone were also found.

In conclusion, the in vivo data indicate and the in vitro data confirm that ketoconazole inhibits cortisol and corticosterone secretion by blocking adrenal 11β-hydroxylase activity in normal subjects as well as in patients with Cushing's syndrome, an effect which is compensated in vivo by high ACTH levels.

Key words: Cortisol secretion – 11β-hydroxylation – Ketoconazole

The chemotherapeutic action of the antifungal agent ketoconazole (Nizoral) is considered to be mediated by inhibition of 14-demethylation of lanosterol, thus reducing the production of ergosterol in fungi [1].

In mammalian cells, ketoconazole also blocks cholesterol synthesis by inhibition of 14-demethylation of the precursor lanosterol [12]. After Pont et al. showed that ketoconazole blunts the serum cortisol response to ACTH in healthy men after a single oral dose, we studied a patient with a cortisol-producing adrenal adenoma and found a pronounced reproducible fall of serum cortisol levels after repeated oral doses of ketoconazole [3]. Furthermore, ketoconazole clearly diminished the cortisol production in tissue slices of the excised adrenal tumor in vitro [3]. In addition, in vivo studies of five patients with Cushing's syndrome (two with an adrenal tumor and three with bilateral adrenal hyperplasia) showed that basal serum cortisol levels were lowered to a variable extent whereas the response to intravenous ACTH was diminished or absent. Incubation studies with adrenal tissue slices of three of these five patients revealed that cortisol levels in the supernatants could be suppressed by 40%–76% in the presence of ketoconazole [4].

Abbreviations: ACTH = Adrenocorticotropic hormone; B = Corticosterone; DOC = 11-Deoxycorticosterone; F = Cortisol; K = Ketoconazole; 17-OH-P = 17α-Hydroxyprogesterone; S = 11-Deoxycortisol
searching for the step where ketoconazole blocks adrenal cortisol biosynthesis, we investigated basal and ACTH-stimulated serum levels of \( F \) and \( B \) as well as of the precursors 17-OH-P, \( S \) and \( DOC \) as well as plasma ACTH levels before and during oral administration of ketoconazole in five patients with Cushing's syndrome and three controls. In addition, the influence of ketoconazole on the transformation of \( ^{3}\text{H}-17\text{-OH-P} \) to \( ^{3}\text{H}-S \) and \( ^{3}\text{H}-F \), of \( ^{3}\text{H}-S \) to \( ^{3}\text{H}-F \), and of \( ^{3}\text{H}-DOC \) to \( ^{3}\text{H}-B \) was examined in slices or homogenates of adrenal tissue from four patients.

**Patients and Methods**

**Patient studies.** The influence of ketoconazole (cis-1-acetyl-4-(4-((2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl)-methoxy)phenyl)piperazine) on basal and ACTH-stimulated serum levels of \( F \) and \( B \) as well as of the precursors 17-OH-P, \( S \) and \( DOC \) and on basal plasma ACTH levels was studied in five patients with Cushing's syndrome (three with bilateral adrenal hyperplasia, one with an adrenal adenoma, and one with an adrenal carcinoma) and in three controls. All patients with Cushing's syndrome had clinical signs of cortisol excess such as truncal obesity, a cervical buffalo hump, chubby cheeks and muscular atrophy of the extremities. All had serum cortisol profiles without diurnal variation (minimal serum levels of greater than 110 ng/ml in the evening). In each case, tissue slices of one patient with Cushing's disease, and the transformation of \( ^{3}\text{H}-S \) to \( ^{3}\text{H}-F \) and of \( ^{3}\text{H}-DOC \) to \( ^{3}\text{H}-B \) in 35 incubation studies with adrenal cortical slices of three patients (1 with adrenal hyperplasia, 1 with pheochromocytoma and 1 with hypernephroma) and with a homogenate from frozen adrenal cortical tissue of one patient with adrenal adenoma. In each case, tissue slices of 100 mg, prepared after the method of Deutsch [2] or homogenates from 100 mg tissue were incubated in 2 ml standard incubation mixture at 37°C for 2 h. The standard incubation mixture contained 151.6 mM NaCl, 4.8 mM KCl, 2.6 mM CaCl\(_2\), 1.2 MgCl\(_2\), 15.7 mM Na\(_2\)HPO\(_4\), 0.294 mM NaD, 0.268 mM NADH, 0.238 mM NADP, 0.986 mM D-glucose-6-phosphate, 5.1 mM D-glucose, and 7 µg D-glucose-6-phosphate-dehydrogenase. All incubations were done in triplicate with or without the addition of 2.5–20 µg/ml ketoconazole. Labelled precursors were also added: \( 5\times 10^{-11} \) mol of \( ^{1,2,3}\text{H}-17\text{-OH-P} \), \( 5\times 10^{-11} \) mol \( ^{1,2,3}\text{H}-S \) or \( 5\times 10^{-11} \) mol \( ^{1,2,3}\text{H}-DOC \). After incubation and homogenisation of the tissue in the medium, the tritium labelled steroids were extracted together with traces of \((4\text{-}^{14}\text{C})\text{-F}\) or \((4\text{-}^{14}\text{C})\text{-DOC}\) for recovery calculations with \( 2\times 10\text{ ml} \) chloroform. After distribution between methanol and \( n\text{-heptane} \) (50:10; \( v:v \)) the steroids were separated by thin layer chromatography using the systems acetone-benzene (1:1; \( v:v \)) as the first system and \( n\text{-heptane} \), benzene, ethylacetate (1:1:4; \( v:v:v \)) as the second system for the incubation experiments with \( ^{3}\text{H}-17\text{-OH-P} \) and \( ^{3}\text{H}-S \) and dichloromethane-ethyl acetate-methanol (85:15:5; \( v:v:v \)) as the third system for the incubation experi-

**Serum assay methods.** The serum levels of \( F \), \( B \), 17-OH-P, \( S \), and \( DOC \) were measured by specific RIA’s after automated Sephadex LH 20 chromatography. The specificity, sensitivity, and precision of the method has been previously published [11]. Plasma levels of ACTH were measured before and during oral ketoconazole by a specific and sensitive RIA after extraction. Lower limit of detection was 5 pg ACTH/ml plasma [6].

**Steroids and chemicals.** Unlabelled \( F \), B, 17-OH-P, \( S \), and DOC were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Tritiated \((1,2,3\text{H})-17\text{-OH-P} \) (specific activity \( 40–60 \text{ Ci/mmol} \)), \((1,2,3\text{H})\text{-S} \) (specific activity \( 40–60 \text{ Ci/mmol} \)), \((1,2\text{H})\text{-DOC} \) (specific activity \( 40–60 \text{ Ci/mmol} \)), \((4\text{-}^{14}\text{C})\text{-F} \) (specific activity \( 50–60 \text{ mCi/ml} \)), and \((4\text{-}^{14}\text{C})\text{-DOC} \) (specific activity \( 50–60 \text{ mCi/ml} \)) were purchased from New England Nuclear Corp., Boston, Mass., USA. Nicotinamide adenine dinucleotide (NAD, disodium salt), nicotinamide adenine dinucleotide, reduced form (NADH, disodium salt), nicotinamide adenine dinucleotide phosphate (NADP, tetra sodium salt), D-glucose-6-phosphate and D-glucose-6-phosphate-dehydrogenase were purchased from Boehringer, Mannheim, West Germany. Thin layer plates (Silicagel 60 F 254) and all solvents were purchased from Merck, Darmstadt, West Germany.