CASE REPORT

Long term control of hypercortisolism with fluconazole: case report and in vitro studies

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Abstract

Objectives: To evaluate the efficacy of fluconazole as an alternative treatment for controlling hypercortisolism in Cushing’s syndrome and to determine its effect on glucocorticoid production in vitro.

Design: Case report and in vitro study in a University Clinic.

Case: An 83 year old patient presented with recurrence of Cushing’s syndrome due to pulmonary metastases three years after unilateral adrenalectomy. During a near fatal episode of sepsis she was started on fluconazole 200 mg/day intravenously which normalised cortisol excretion. The therapy was continued orally for 18 months. Upon temporary discontinuation and reintroduction of treatment, cortisol levels increased and normalized, respectively. At month 16, fluconazole had to be increased to a dose of 400 mg/day to keep cortisol excretion in the normal range. Disease progression was slow and no side effects occurred.

In vitro results: Fluconazole in a concentration of 500 \( \mu \text{M} \) nearly abolished corticosterone production over 24 h from the adrenal adenoma cell line Y-1 (8.6\(^\pm\)0.5% compared with control, \( P < 0.0001 \)) and significantly reduced corticosterone production in concentrations of 50 \( \mu \text{M} \) (48.3\(^\pm\)1.9% vs. control, \( P < 0.0001 \)) and 5 \( \mu \text{M} \) (80.5\(^\pm\)8.5% vs. control, \( P < 0.05 \)).

Conclusion: These results demonstrate for the first time that fluconazole normalises cortisol concentrations in vivo in a patient with Cushing’s syndrome with adrenal carcinoma and inhibit glucocorticoid production in vitro in a cell line. Thus, fluconazole might be useful in controlling glucocorticoid excess in Cushing’s syndrome and because of its lower toxicity might be preferable to ketoconazole.

Introduction

Surgical excision of an adrenal adenoma or carcinoma is the primary therapy for Cushing’s syndrome. If the patient cannot be cured by surgery, inhibitors of steroidogenesis are the treatment of choice (1, 2). Many studies have reported that ketoconazole can effectively control hypercortisolism and therefore it is considered as first line therapy in surgically uncurable Cushing’s syndrome (2–6). It is surprising that the literature is devoid of any data on fluconazole in this indication. Fluconazole, like ketoconazole, is an antifungalazole-derivative. It is better tolerated than ketoconazole and considered less toxic (7–10). Most reports claim that fluconazole does not suppress adrenal cortisol synthesis in doses up to 400 mg per day even when administered for several weeks (8–12). However, anecdotal reports indicate that fluconazole indeed might suppress adrenal steroidogenesis (13–15) but this has not been studied systematically and occasional reports in critically ill patients might be of limited relevance to otherwise healthy subjects with Cushing’s syndrome.

The present report documents the long-term effect of fluconazole in controlling cortisol excess in a patient with pulmonary metastases due to an adrenal glucocorticoid-producing tumor operated on 3\( \frac{1}{2} \) years before. In addition, in vitro effects of fluconazole were studied in an adrenal corticosterone producing rat cell line (Y-1).

Patient and methods

Case report

A 78-year-old female presented in 2000 with the full clinical picture of Cushing’s syndrome and a history of osteoporosis, bone fractures (hip and ankle) and gastric ulcer. The work-up revealed Cushing’s syndrome due
to an adrenal tumor of the right gland. Right adrenalectomy was performed in August 2000 and histologically classified as adrenal adenoma. Thereafter, the patient exhibited clinical signs and laboratory evidence of adrenal insufficiency necessitating glucocorticoid substitution therapy for more than one year. In November 2003 the patient again noticed symptoms of Cushing’s syndrome (weight gain with central obesity, muscle wasting, hypertension) and this was confirmed by elevated urinary cortisol excretion and the failure to suppress serum cortisol concentration after 1 mg dexamethasone. Radiological evaluation revealed a normal abdominal MRI, however, multiple lung nodules were seen in the chest CT-scan. At that time the patient underwent a near-fatal episode of sepsis due to spondylodiscitis (L2–L5) and was started on 200 mg fluconazole (Pfizer PGM, Poce-sur-Cisse, France) intravenously, since ketoconazole is not available in parenteral form. The patient responded promptly with suppression of cortisol production (Table 1), urinary cortisol excretion dropped from 295 μg/24 h to 109 μg/24 h and her general condition improved significantly. She underwent laminectomy and could be successfully mobilised thereafter. Because of good control of hypercortisolism with an oral dose of 200 mg fluconazole, the lack of any side effects and the obviously slow progression of disease (pulmonary micrometastases must have been present at the time of adrenalectomy in 2000) therapy was continued with regular controls every 2–3 months. Although cortisol excretion and morning serum cortisol concentrations do not ideally reflect the activity of the pituitary–adrenal axis they could easily be monitored in the patient’s follow-up and were paralleled by clinical parameters such as hypertension, need for antihypertensive therapy and HbA1C.

Nine months after diagnosis of Cushing’s syndrome recurrence, persistent normal cortisol excretion with 200 mg fluconazole and only slow tumor growth (Fig. 1a) fluconazole was temporarily discontinued and an 11C metomidate PET scintigraphy (Fig. 1b; 16) was performed to prove that the pulmonary nodules were the source of excessive cortisol production since no histology could be obtained at the time of manifestation. The cessation of fluconazole resulted in an increase in cortisol excretion above pre-treatment values and normalization after reinstitution of therapy (Table 1). Sixteen months after disease recurrence, the dose of fluconazole had to be increased to 400 mg/day because of increasing cortisol excretion. At 18 months after institution of fluconazole therapy, the patient was still well controlled and at good general condition considering her age of now almost 83 years.

The patient tolerated the drug well and safety parameters such as liver function tests, serum aminotransferases and blood count remained unchanged over the whole observation period of 18 months (data not shown).

Table 1 Urinary cortisol excretion, serum cortisol concentrations and clinical parameters before and during therapy with fluconazole in a 83 year old patient with Cushing’s syndrome.

<table>
<thead>
<tr>
<th>Time course of fluconazole therapy</th>
<th>Start fluconazole 200 mg</th>
<th>Discontinuation fluconazole</th>
<th>Reinstitution fluconazole 200 mg</th>
<th>Start fluconazole 400 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12/03</td>
<td>1/04</td>
<td>4/04</td>
<td>7/04</td>
</tr>
<tr>
<td>Urinary cortisol excretion (μg/24h)</td>
<td>295.7</td>
<td>109</td>
<td>21.6</td>
<td>38.2</td>
</tr>
<tr>
<td>Morning serum cortisol concentration (μg/dl)</td>
<td>19.4</td>
<td>13</td>
<td>20.3</td>
<td>19.4</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>100</td>
<td>141</td>
<td>93</td>
<td>91</td>
</tr>
<tr>
<td>HbA1C (normal range – 6.0%)</td>
<td>5.9</td>
<td>6.0</td>
<td>5.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Blood pressure (systolic/diastolic mmHg)</td>
<td>160/90</td>
<td>n.a.</td>
<td>125/80</td>
<td>120/80</td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spironolactone(mg)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Hydrochlorothiazide(mg)</td>
<td>12.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

n.a.; not available; urinary cortisol excretion normal range 36–137 μg/124 h; fasting blood glucose normal range 76–100 mg/dl.
In vitro studies

The rat adrenocortical cell line (Y-1, no. ccl-79 ATCC, Monassas, VA, USA) was used to assess the effect of fluconazole (Pfizer PGM) on glucocorticoid production and to compare it with ketoconazole (Sigma, St. Louis, MA, USA). Cells were seeded at a density of $5 \times 10^5$ cells/ml in 12-well plates and allowed to attach overnight. Medium was then replaced with substances added. Cells were incubated for 3 or 24 h at 37°C, 6% CO$_2$, 95% relative humidity. Supernatant was transferred to cryovials containing 0.1% EDTA and stored at $-30^\circ$C. Incubations were done in triplicates. Incubation with ACTH (1 $\mu$M) served as positive control and incubation with culture medium alone served as negative control in each experiment. Corticosterone concentration in the cell culture supernatant was determined by RIA. Data from at least seven wells from three independent experiments are presented as mean (±S.E.M.) corticosterone concentrations (ng/well). Differences between fluconazole or ketoconazole treatment and control were compared using student’s $t$-test. Effects of different ketoconazole or fluconazole concentrations were compared with control with one-way ANOVA followed by Dunnett-T3 as post-hoc statistics. SPSS 12.0 was used as statistical software. Significance level was set at $P<0.05$.

Results

The effects of ketoconazole and fluconazole on corticosterone release after 3 h incubation are shown in Fig. 2a and 2b, and after 24 h incubation in Fig. 2c and 2d. Both azole-derivatives reduced basal corticosterone release significantly at a concentration of 5 $\mu$M, but ketoconazole had a significantly greater effect than fluconazole (Fig. 2a and 2c). Similar results were obtained when the effects on ACTH-induced corticosterone release were tested: ketoconazole inhibited corticosterone release to a greater degree than fluconazole after 3 h incubation (Fig. 2b). After 24 h, ketoconazole almost completely abolished corticosterone release while fluconazole at the same concentration had no significant effect (Fig. 2d).

In the dose–response experiments, 24 h corticosterone production was almost completely suppressed at a concentration of 5 $\mu$M ketoconazole while fluconazole at the same concentration exhibited only a small effect (Fig. 3). However, when fluconazole was added to Y-1 cells at a concentration of 50 $\mu$M, corticosterone production was suppressed to less than 50% of untreated cells and to less than 10% by 500 $\mu$M fluconazole.

Discussion

This case report documents for the first time that hypercortisolism of Cushing’s syndrome due to an adrenal carcinoma can be effectively and safely treated with the antifungal azole-derivative fluconazole over a period of 18 months. We neither observed side effects at a dose of 200 mg fluconazole per day nor did we when the dose was increased to 400 mg/day. Lower toxicity of fluconazole compared with ketoconazole has been
demonstrated in several in vitro studies. In a primary rat hepatocyte culture system allowing the assessment of plasma membrane integrity and mitochondrial as well as lysosomal stability fluconazole was shown to exert considerably less cytotoxic effects than ketoconazole (17). Similar results were also seen in human granulocyte-macrophage progenitor cells in vitro (18).

Fluconazole therapy was started in this patient in order to control hypercortisolism in a situation of life-threatening sepsis. Due to the good effect, lack of side effects and slow progression of disease, we decided to continue the therapy because therapeutic alternatives such as mitotane were expected to impair the quality of life of this now 83 year old lady to a significantly greater extent. Noteworthy, we have observed a reduction of glucocorticoid production also in another patient with ectopic Cushing’s syndrome due to an unidentified tumor (M Riedl, C Maier and A Luger, personal observation). However, after a good initial response (66% reduction) cortisol excretion increased again and could not be normalised with 400 mg fluconazole indicating that fluconazole might not be suited to treat ACTH-dependent Cushing’s syndrome. Bilateral adrenalectomy was performed after 3 months of therapy in this patient.

The good in vivo effect of fluconazole could be supported by in vitro data. Although ketoconazole was effective at much lower concentrations, fluconazole inhibited corticosterone production by Y1 cells significantly. Noteworthy, the effective concentrations of the in vitro experiments are in the range of the therapeutic plasma concentrations (19). Eckhoff et al. (20) also have reported a potent inhibitory effect of ketoconazole on steroidogenesis but only a weak effect of fluconazole (less than 50% inhibition at 10⁻³ M fluconazole) in a primary rat cell culture system. The fact that we registered a significant inhibitory effect of fluconazole at lower doses than previously described might be

**Figure 2** Effect of ketoconazole (keto) and fluconazole (flu) at a concentration of 5 µM on (a) basal corticosterone release over 3 h, (b) ACTH (1 µM) stimulated corticosterone release over 3 h, (c) basal corticosterone release over 24 h and (d) ACTH (1 µM) stimulated corticosterone release over 24 h in Y1 cells. co, corticosterone concentration in supernatant of unstimulated cells after 3 or 24 h respectively (negative control); ACTH, corticosterone concentration in supernatant of ACTH (1 µM) stimulated cells after 3 or 24 h respectively (positive control). *P < 0.05, **P < 0.01, ***P < 0.0001.
attributed to the different experimental procedures, i.e. primary non-tumor cell culture vs. tumor cell line. The different plasma protein binding properties of the two substances (ketoconazole 99%, fluconazole 11%; 7,8) might in part be responsible for their almost equipotent effects in humans in vivo. In fact, even a reversed order of inhibitory potencies on cytochrome P-450-dependent drug metabolism has been reported for ketoconazole and fluconazole in vivo (21). In addition, bioavailability of fluconazole is greater than of ketoconazole (7).

In a previous report (11), where adrenal function was tested in a small group of patients receiving 400 mg fluconazole/day for two weeks, normal cortisol responses to 250 μg ACTH was observed in all patients. In another randomized, double blind, placebo-controlled study on the effect of fluconazole (400 mg/day) for prevention of candidiasis, mean plasma cortisol concentrations were not different between patients receiving fluconazole or placebo (12). In addition, duration of fluconazole therapy was not associated with adrenal dysfunction. Nevertheless, a considerable number of patients had adrenal dysfunction in this study but clinical outcome was not different in patients receiving placebo or fluconazole. Large reviews on antifungal agents (8, 9) also state that in contrast to ketoconazole, fluconazole does not interfere with adrenocortical function. In contrast, three case reports (on a total of seven patients) found adrenal dysfunction after fluconazole ranging from mild impairment to severe insufficiency (13–15). However, a clear distinction, whether the underlying disease or treatment with fluconazole was the major contributor to adrenal dysfunction could not be made.

While most reports show no or only slight impairment of glucocorticoid production with standard antifungal doses in patients with normal adrenal function (11, 12), profound suppression of cortisol production at the same dose was observed in the patient reported here. This might be related to different expression of steroidogenic enzymes in tumor cells (22–24). In accordance with the experience with ketoconazole in patients with Cushing’s syndrome in the present patient, hypercortisolism could be controlled with 200 mg fluconazole, a dose widely used when the drug is employed as antymycotic therapy. This first clinical observation needs to be confirmed in additional patients.

In summary, this case report and the in vitro data suggest that fluconazole might be effective in suppressing enhanced glucocorticoid production in Cushing’s syndrome due to an adrenal carcinoma. Due to the reported lower incidence of the sometimes serious side effects fluconazole might prove superior to ketoconazole in this indication.

References

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