Pituitary (2014) 17:109–117
DOI 10.1007/s11102-013-0475-3

Combination therapy for Cushing's disease: effectiveness of two schedules of treatment. Should we start with cabergoline or ketoconazole?

M. Barbot · N. Albiger · F. Ceccato · M. Zilio · A. C. Frigo · L. Denaro · F. Mantero · C. Scaroni

Published online: 7 March 2013

Abstract Cushing's disease (CD) is associated with increased morbidity and mortality. Until now, no medical treatment has been shown to be totally satisfactory when administrated alone. This study aimed to assess the effectiveness of cabergoline with added ketoconazole and of the same combination in reverse, using urinary free cortisol (UFC) and late night salivary cortisol (LNSC) levels as biochemical markers of the treatments' efficacy in CD patients. A prospective analysis conducted on 14 patients (f/m = 12/2; median age 52, range 33–70 years) divided into two groups: 6 patients initially treated with cabergoline for 4–6 months (rising from 0.5–1 mg/week up to 3.0 mg/week), after which ketoconazole was added (group A); and 8 patients first took ketoconazole alone for 4–6 months (rising from 200 mg/day to 600 mg/day), then cabergoline was added (group B). Patients were compared with 14 age-matched patients in prolonged remission after effective neurosurgery for CD. The combination therapy led to UFC normalization in 79 % of patients with no differences between the groups; only one patient failed to respond at all. Neither drug succeeded in controlling the disease when taken alone. LNSC dropped when compared to baseline levels, but not to a significant degree ($p = 0.06$), and it remained significantly higher than in controls ($p = 0.0006$). Associating cabergoline with ketoconazole may represent an effective second-line treatment, achieving a satisfactory reduction in UFC levels and clinical improvement. Although the combined treatment lowered patients' LNSC levels, they remained higher than normal, indicating a persistent subclinical hypercortisolism; the implications of this condition need to be considered. No differences emerged between the two treatment schedules.

Keywords Cushing's disease · Ketoconazole · Cabergoline · Late night salivary cortisol

M. Barbot (✉) · N. Albiger · F. Ceccato · M. Zilio · F. Mantero · C. Scaroni
Endocrinology Unit, Department of Medicine DIMED, University of Padova, Via Ospedale Civile, 105, 35128 Padova, Italy
e-mail: mattiabarbot@alice.it

A. C. Frigo
Department of Cardiac, Thoracic and Vascular Sciences, University of Padova, Padua, Italy

L. Denaro
Neurosurgical Unit, Neuroscience Department, University of Padova, Padua, Italy

Introduction

Cushing's disease (CD) due to an ACTH-secreting pituitary tumor is associated with increased morbidity and mortality rates, so timely and definitive treatment is mandatory. The first choice for treating CD is transsphenoidal adenomectomy: this is effective in about 60–80 % of cases depending on the operator's skill. However the not negligible percentage of patients with persistent disease and the risk of recurrence that may exceed 20 % of previously-cured patients in the long term, adding to the number of patients who need second-line treatment to control their hypercortisolism [1–5]. The other possible treatments are pituitary radiotherapy and bilateral adrenalectomy, but they are associated with significant lifelong complications [6]. Hence the need for a genuinely effective medical approach to contain cortisol levels while patients await surgery or the effects of radiotherapy, or when patients refuse surgery or it is contraindicated. Several

medical therapies including neuromodulatory medications and adrenal blocking agents have been proposed, but only the somatostatin analog pasireotide has recently been approved for the treatment of CD. The first drug successfully used to control CD was ketoconazole, which blocks cytochrome P450 enzymes, reducing cortisol secretion by the adrenal glands [7, 8]. Unfortunately, it frequently fails to control hypercortisolism, however, either because ACTH overrides its cortisol-blocking action or due to intolerable side effects. The discovery of dopamine receptor type 2 (D2) expression in corticotroph tumors [9], and the in vitro and in vivo demonstration that dopamine agonists can reduce ACTH secretion led to introduction of cabergoline in the treatment of CD, which proved successful in approximately 40 % of patients. More recently, combination therapies in which ketoconazole is added to cabergoline have been found to increase the numbers of patients responding to each of the drugs when taken alone [10]. The rationale behind this approach would be that using drugs with complementary pharmacological mechanisms offers a higher chance of hypercortisolism being controlled in the long term, using lower doses of each drug and consequently with a lower incidence of side effects. There are no reports on the reverse combination, adding cabergoline to ketoconazole, a choice that may be justified by the initial effect of ketoconazole in reducing cortisol levels possibly enabling a more pronounced subsequent effect of cabergoline on ACTH secretion. The aim of our study was to compare the effectiveness of such combined therapies (cabergoline with added ketoconazole, or ketoconazole with added cabergoline) at relatively low doses and using different schedules, by analyzing their effects on hormonal and clinical parameters.

Patients

This prospective study involved 14 patients (f/m = 12/2; median age 52, range 33–70 years) with persistent (6 cases) or recurrent (4 cases) CD after surgery, or without prior surgery (4 cases). Medical therapy was used as the first choice when patients refused surgery or had severe comorbidities that contraindicated surgery. Patients were enrolled from among the outpatients attending the Endocrinology Unit at the University of Padua; they all signed an informed consent to take part in this study. The study was designed in accordance with the Declaration of Helsinki and approved by our local Ethical Committee. The diagnosis of CD was suspected on clinical features and confirmed by at least two tests indicating high 24 h urinary free cortisol (UFC) levels, loss of circadian rhythm in plasma or salivary cortisol (at 0800 and 2300 h), and lack of cortisol suppression after 1 mg of dexamethasone overnight (normal values $\leq$1.8 mg/dl). The diagnosis of ACTH-dependent syndrome was confirmed by detectable ACTH levels, a decrease in cortisol $\geq$80 % of the baseline value after the overnight 8 mg dexamethasone challenge, and an increase in ACTH ($\geq$50 %) and cortisol ($\geq$20 %) in the CRH tests. All patients had pituitary magnetic resonance imaging (TESLA 1.5) and some also underwent bilateral inferior petrosal sinus sampling (BIPSS) when

Table 1 Patients' baseline characteristics

Patients	Sex/age	BMI (kg/m^2)	Previous TSS	MRI	UFC (mmol/24 h)	LNSC (ng/ml)	ACTH (pg/ml)	PRL (μg/l)
Treated at first with cabergolin GROUP A								
1	f/55	25.2	TSS	neg	1,404	16.40	62	10.3
2	f/54	23.8	TSS	m	1,274	8.83	36	20.5
3	f/65	39.2	none	m	1,342	6.50	18	15.4
4	f/65	32.5	TSS	m	1,515	6.70	41	25.5
5	f/50	26.6	TSS	m	800	24.10	28	–
6	f/37	25.5	TSS	m	980	6.68	39	19.2
7	f/65	33.3	TSS	in	1,243	13.50	60	7.7
Treated at first with ketoconazole GROUP B								
8	m/52	25.9	none	m	2,789	21.10	89	32.4
9	f/72	41.6	none	neg	1,118	6.75	15	19.5
10	f/50	40.9	TSS	in	971	8.61	41	14.4
11	m/39	29.6	TSS	neg	973	15.05	15	20.2
12	f/43	23.9	none	M	2,191	11.92	16	17.3
13	f/64	27.9	TSS	neg	1,021	5.05	16	10.2
14	f/55	22.8	TSS	neg	1,067	12.00	25	9.1

Normal ranges for: prolactin, 5–25 μg/l; UFC, 90–694 nmol/24 h; LNSC, <3.5 ng/ml

TSS transsphenoidal surgery; *PRL* prolactin; *neg* negative; *m* microadenoma; *M* macroadenoma

biochemical hormone test findings were not consistent with a pituitary origin of the ACTH secretion. The diagnosis of persistent or recurrent CD was based on the detection of high UFC levels, loss of diurnal rhythm and cortisol suppression after 1 mg dexamethasone. All patients had high UFC levels, which ranged from 1.2 to 4.0 times the upper limit of normality (normal range 90–694 nmol/24 h). Two patients had mildly elevated prolactin (PRL) levels. One had a macroadenoma that was treated with medication while surgery was delayed due to a severe joint infection with systemic involvement. The patients' characteristics are summarized in Table 1.

Fourteen age-matched patients (11 females and 3 males; median age 42 years, range 26–71) in long-term remission of Cushing's disease (from 29 to 122 months) were studied as controls, measuring their UFC and salivary cortisol at 23.00 h (LNSC); none of the controls were taking corticoid replacement therapy (Table 2).

Materials and methods

The patients were randomly assigned to group A and given cabergoline alone (0.5–1 mg/week), or to group B and treated with ketoconazole (200 mg/daily) alone. The dosage of both drugs was gradually increased: the cabergoline dose rose by 1 mg every month until UFC levels normalized or up to a maximal dose of 3.0 mg/week; ketoconazole by 200 mg/day every month and titrated up until UFC normalization or to a maximal dose of 600 mg/day. When UFC failed to reach 30 % of reduction, ketoconazole 200 mg/day was introduced in Group A, increasing the dose (200 mg/day every month) until normalization of UFC or when a dose of 600 mg/day was reached. The same was done for the Group B where cabergoline was introduced and increased until UFC normalized or to a maximum dose of 3.0 mg/week (Fig. 1). We monitored aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GT) every fortnight for the first 2 months, then monthly. A clinical assessment was performed once a month. After 6 months of monotherapy, if neither UFC nor LNSC were under control, ketoconazole was added in group A, and cabergoline in group B. Patients' clinical features before starting the medical treatment (signs of hypercorticolism, BMI, waist circumference and blood pressure) and biochemical test results (morning fasting glucose, glycosylated hemoglobin HbA1c, PRL, basal ACTH, 24-h UFC, and LNSC) were recorded and compared with post-treatment values in all cases. We also compared pituitary MRI before and after therapy in patients with a longer follow-up.

Table 2 Controls' characteristics

Patients	Age	Sex	Time after surgery (months)	UFC (nmol/24 h)	LNSC (ng/ml)
RE1	26	m	102	492	2.39
RE2	63	m	14	212	3.3
RE3	61	f	82	485	2.75
RE4	39	f	33	381	2.11
RE5	41	f	28	248	0.12
RE6	39	f	137	309	1.37
RE7	47	f	31	548	2.15
RE8	39	f	15	333	2.28
RE9	60	f	71	652	2.4
RE10	44	m	122	322	1.15
RE11	35	f	39	331	0.3
RE12	71	f	14	189	2.16
RE13	33	f	84	419	0.8
RE14	45	f	80	330	1.9

Normal ranges for: UFC 90–694 nmol/24 h; LNSC <3.5 ng/ml

Saliva sample collections and biochemical assays

Patients collected saliva samples late at night, before going to bed. Each saliva aliquot was collected in cotton-based sampling devices and stored in plastic syringes using the Salivette® commercial kit (Sarstedt, Numbrecht, Germany). Patients were asked to soak the absorbent cotton with saliva for 2 or 3 min, then place the sample in the syringe and keep it in their refrigerator. Samples were collected at least 30 min before or after eating or drinking to avoid any source of food or blood contamination; patients brushed their teeth at least 30 min before collecting their saliva and avoided smoking or eating liquorice on the day of saliva collection. After centrifugation, saliva samples were stored at −20 °C until cortisol analyses were performed with the RIA kit (Radim, Rome, Italy) with an assay sensitivity of 0.5 ng/ml; 24 h UFC was measured by RIA (normal range 90–694 nmol/24 h), considering the mean of at least two collections, using a commercial kit (Biodata Diagnostic, Rome, Italy) with a sensitivity of 8 nmol/l; ACTH at 8.00 h (normal range 5–50 pg/ml) was tested by IRMA (Nicholas Institute Diagnostics, San Juan Capistrano, CA, USA) with an assay sensitivity of 1.2 pg/ml. The intra- and inter-assay coefficient of variation for all assays used in the study were <7 and 9 %.

Statistical analysis

Quantitative data are given as means and standard deviations, or medians and ranges, and were compared between groups using Wilcoxon's rank sum and Wilcoxon's test followed by the Dunn test for pairwise comparisons in the event of a statistically significant result when more than two groups were involved. Categorical data are summarized as

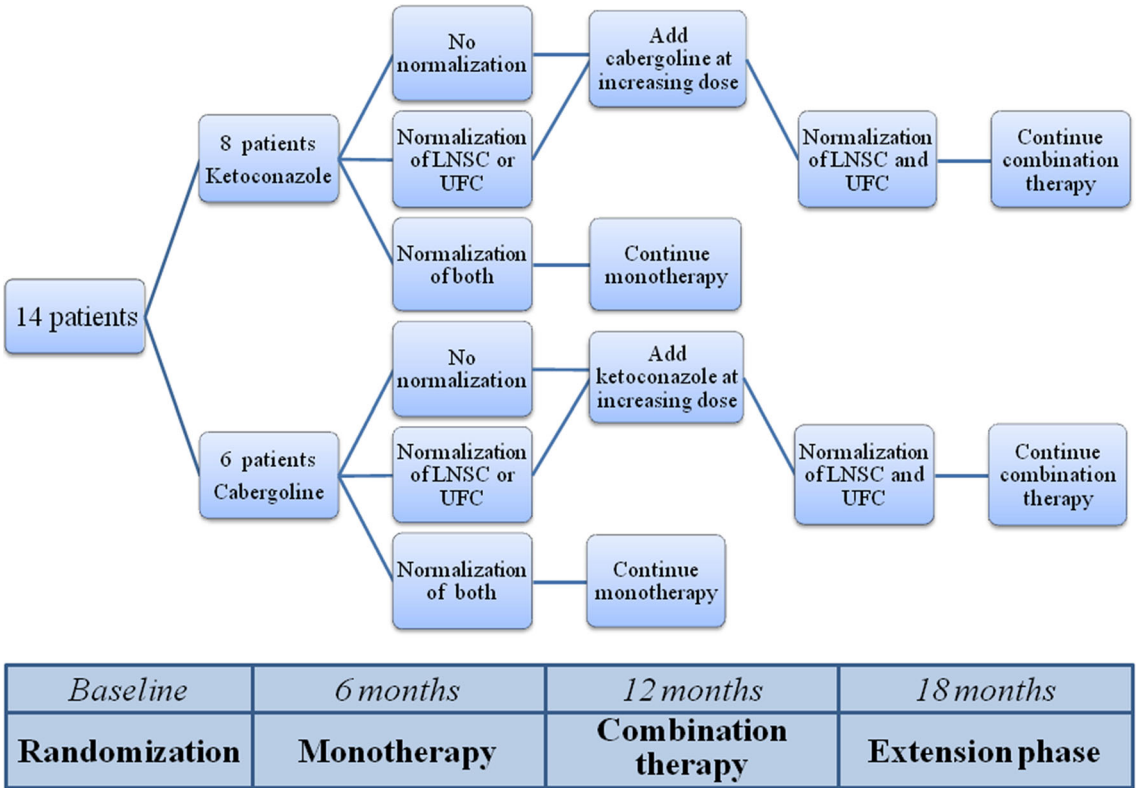

Fig. 1 Flow chart of the treatment 2 treatment schedules; all but one patient, who had to stop the medical therapy because of no response, were treated for at least 12 months. Four patients with both UFC and LNSC within normal range, continued the combination therapy for other 6 months

counts and percentages of subjects in each category and were compared using the Chi square or Fisher's exact test. Statistical significance was set at a p value of less than 0.05. Data were analyzed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). With a sample size of 14 patients, we could find statistically significant an effect size of 0.9 with alpha set at 5 % and power at 80 % in a paired comparison; with regards to the comparison between groups, with 6 and 8 patients in each respectively, we could declare statistically significant an effect size of 2 with alpha set at 5 % and power at 80 % (non parametric test, PASS 11 for Windows, NCSS, LLC, Kaysville, Utah, USA).

Results

There were no significant differences between groups A and B in terms of age, sex, clinical features or initial hormone levels. In group A (6 patients initially treated with cabergoline), two patients (33 %) achieved UFC normalization after cabergoline therapy alone (one was on 2 mg and the other on 3 mg/week), but their LNSC did not return to normal during the 6 months of treatment. One case in this group had a partial response with a more than 30 % decrease in the patient's baseline UFC levels (partial responder). Another 2 patients had a decrease of 24 and 21 % in their UFC levels. The last patient in group A was unresponsive to cabergoline, so we introduced ketoconazole earlier than in the other patients.

In group B (8 patients given ketoconazole as first-line therapy), we observed a complete and prolonged response in terms of UFC in 5 patients on low doses of ketoconazole, and a significant reduction in the UFC levels in the other 3, though they did not return to normal. Four of the former 5 patients remained in a eucortisolic state for 6 months at the same drug dosage, while ketoconazole had to be reduced in one due to high liver enzymes, and in another due to skin rash, with a consequent increase in cortisol secretion. After 6 months, none of our patients had both UFC and LNSC within the normal range, so the combined therapy was started. In group A, adding ketoconazole prompted a decrease in UFC levels, leading to 83 % of complete responders and one patient with a partial response (Fig. 2). In group B, adding cabergoline normalized the UFC level in one patient and improved UFC control in another 3 (Fig. 2). Only one patient in group B failed to respond to cabergoline, with a rise in UFC to 3886 nmol/24 h. The overall success rate for the combined therapies, considering

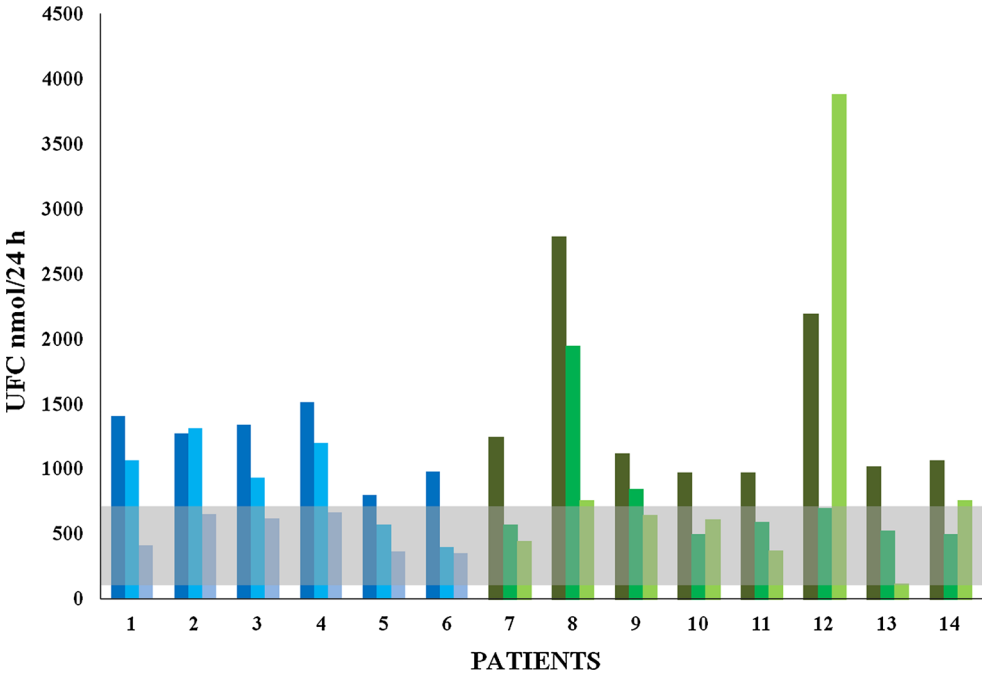

Fig. 2 UFC levels at baseline (*darker bars*), on monotherapy (*intermediate bars*) and on combined therapy (*lighter bars*); the range of *blue* was used for patients initially treated with cabergoline; range of *green* colors for patients initially treated with ketoconazole. The gray stripe represents the range of normality (90–694 nmol/24 h)

UFC levels alone, was 79 %, and these results persisted for at least 6 months. Of the 3 patients whose UFC was not controlled, 2 achieved a drop in UFC levels (−79 and −29 % vis-à-vis the baseline) with improvements in their clinical signs. None of the parameters assessed at the baseline were able to predict response to the combined therapy, though patients with high UFC levels at the baseline (more than twice the upper limit of normality) tended to have a less satisfactory response ($p = 0.066$). As for LNSC, there was a slight drop, but it was not statistically significant (baseline vs monotherapy $p = 0.52$, monotherapy vs combined therapy $p = 0.15$, baseline vs combined therapy $p = 0.06$), table 3. In the majority of cases (10/14), this marker remained above the normal range for our laboratory (normal values <3.50 ng/ml) even in patients whose UFC levels normalized (Fig. 3). When patients with a history of pituitary surgery were compared with patients who had *de novo* CD, the efficacy of this combined medical treatment in normalizing UFC secretion was lower in the latter group than in the former ($p = 0.04$), while no differences emerged as regards reductions in LNSC ($p = 0.12$). This difference in UFC levels between these two groups was found also at baseline ($p = 0.05$).

All 14 controls (patients in prolonged remission of CD) had normal UFC levels (median 332; range 189–652 nmol/24 h) and LNSC (median value 2.13, range 0.12–3.30 ng/ml).

Table 3 Biochemical and clinical features of the 14 patients with CD at baseline (basal), on cabergoline or ketoconazole monotherapy (mono) and on combination therapy (comb)

	Basal	Monotherapy	Combined therapy	*p* value		
				Mono versus basal	Comb versus basal	Mono versus comb
UFC (nmol/24 h)	1180.5 (800–2,789)	646.5 (398–1,944)	617 (121–3,884)	0.0006	0.03	ns
LNSC (ng/ml)	10.38 (5.05–24.1)	8.34 (2.79-14.0)	6.28 (1.62-12.0)	ns	ns	ns
ACTH (pg/ml)	32 (15–84)	34.5 (15–84)	35 (12–76)	ns	ns	ns
Glycemia (mmol/l)	5.3 (4.1–1.8)	5.2 (3.9–11)	5.0 (3.2–8.9)	ns	ns	ns
HbAlc (mmol/mol)	54 (38–114)	51 (38–92)	44 (37–78)	ns	0.045	0.02
BMI (kg/m^2)	27.25 (22.8–41.6)	27.95 (22.8–42.4)	25.7 (23.2–40.5)	ns	ns	0.018
Waist (cm)	106 (78–126)	99 (75–125)	98.5 (76–126)	0.02	0.006	ns

Values are expressed as medians and ranges

Normal ranges for: UFC 90–694 nmol/24 h; LNSC <3.5 ng/ml; ACTH 10–50 pg/ml

When compared with the patients on medical treatment as a whole (groups A + B), they had lower UFC ($p = 0.01$) and LNSC levels ($p = 0.0006$), but when controls were compared with complete responders (11/14 treated patients; median UFC 447, range 121–668 nmol/24 h), there were no differences in terms of their UFC levels ($p = 0.07$), whereas the LNSC levels remained higher (median 5.64, range 1.62–10.4 ng/ml) in the patients on medical treatment ($p = 0.003$). In the 4 patients with normal LNSC the treatment was extended for another 6 months: this led to a gradual increase in LNSC to pathological values in 3 cases, while UFC levels remained under control (albeit closer to the upper limit of normality) in all patients. When MRI was performed in 10 patients at the end of the treatment, there was no difference in the size of their pituitary lesions with respect to images obtained beforehand.

As for their clinical features, most of our patients showed a partial regression in the signs of hypercortisolism, including reductions in waist circumference and BMI (Tables 3, 4), and a drop in the number and/or dose of antihypertensive drugs taken by complete and partial responders, and this specific treatment was completely withdrawn in two cases. It is worth noting that this favorable effect was more evident in patients whose LNSC was normal while they were on combined therapy. The 6 diabetic patients (all controlled with the combined therapy) had a better metabolic control, as measured from fasting glucose and HbA1c levels: the drop in HbA1c ranged from 0.3 to 3.2 % after 6 months of combined therapy. At the end of the study all patients were taking both medications, the mean dosage was 2.3 mg/week of cabergoline and 314 mg/day of ketoconazole.

Side effects

Both treatments were well tolerated and no severe side effects were reported. Ketoconazole was reduced from 400 to 200 mg/day in 2 patients in group B, due to high transaminases in 1 case and to skin rash in the other; the symptoms regressed completely with this dose reduction. None of the patients experienced hypoglycemia, hypotension or adrenal insufficiency while on mono- or combined therapy. Echocardiograph surveillance was performed in the 8 patients (6 of group A and 2 of group B) who were taking cabergoline for more than one year. No tricuspidalic regurgitation was observed in these patients.

Discussion

Cushing's disease is a rare disorder coinciding with increased morbidity and mortality rates; a rapid reversal of this state is mandatory to limit the side effects of hypercortisolism [11, 12]. In persistent or recurrent CD, second-line treatments have to be considered, the possible options being repeat surgery, pituitary irradiation, bilateral adrenalectomy or medical therapy. Unlike other pituitary adenomas, such as acromegaly and prolactinomas, there is currently no effective medication that takes action specifically on corticotroph tumor [10, 13–15]. The somatostatin analog pasireotide showed positive results both in the short

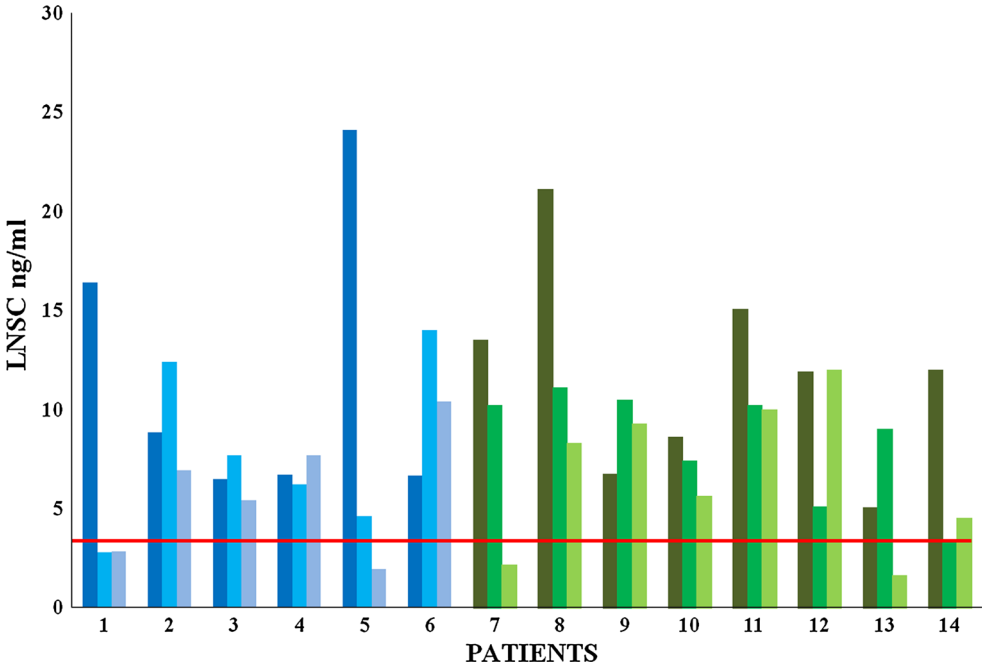

Fig. 3 LNSC levels at baseline (*darker bars*), on monotherapy (*intermediate bars*) and on combined therapy (*lighter bars*); the range of *blue* was used for patients initially treated with cabergoline; range of *green* colors for patients initially treated with ketoconazole. The red line represents the upper limit of normality (< 3.5 ng/ml)

term and after a year of treatment for CD in approximately 30 % of patients and has recently been approved by the European Medicine Agency for treatment of Cushing's disease in patients in whom surgery has failed or is not a viable option [16–18]. Promising results obtained in vitro with retinoic acid were confirmed in vivo by a recent pilot study, suggesting a role for this drug in the treatment of CD [18, 19]. Other neuromodulatory drugs were used in the past. First, bromocriptine was reported to induce a lower cortisol secretion, but eucortisolism and tumor shrinkage were rarely maintained by long-term treatment [20–22]. Better results were obtained with the more potent dopamine agonist, cabergoline, which is also longer-acting and associated with fewer side effects than bromocriptine [23, 24]. Response to dopamine agonists depends on D2 receptor expression and activity, which reportedly varies from 51 to 75 % in both complete and partial responders [9, 13, 25, 26].

Cabergoline monotherapy

In our series, 6 patients were given cabergoline as first-line medical treatment, but only 3 of them were considered responders. Some patients' failure to respond to this treatment may be due to their having little or no D2 receptor expression in the tumor cells, or to a downregulation of these receptors by persistently high cortisol levels. Only 2 of our patients had mild hyperprolactinemia at the baseline, which had been associated (at least to some degree) with responsiveness to cabergoline treatment: Pivonello et al. [13] reported a better response to cabergoline than we found, possibly because 45 % of their patients had mild hyperprolactinemia as well, and because they used a higher dose of dopamine agonist (7 mg/week). Response to cabergoline became evident in our patients at lower doses (2–3 mg/week) and then we opted to add the other drug.

Table 4 Comparison between the two groups at baseline (basal), on monotherapy (mono) and on combination therapy (combined)

Parameters	Group A	Group B	*p* value
UFC (nmol/24 h)			
Basal	1,308 (800–1,515)	1,092 (971–2,789)	0.85
Mono	1,001.5 (398–1,311)	5 82.5 (497–1,944)	0.33
Combined	515 (354–668)	628.5 (121–3,884)	0.48
LNSC (mg/ml)			
Basal	7.77 (6.5–24.1)	11.96 (5.05–21.1)	0.75
Mono	6.94 (2.79–14)	9.62 (3.4–11.1)	0.85
Combined	6.16 (1.93–10.4)	6.97 (1.62–12)	0.75
ACTH (pg/ml)			
Basal	38.5 (18–62)	21 (15–89)	0.36
Mono	41 (19–55)	27.5 (18–84)	0.95
Combined	35.5 (28–71)	32 (12–76)	0.52
BMI (kg/m^2)			
Basal	26.1 (23.8–39.2)	28.8 (22.8–41.6)	0.65
Mono	25.6 (24.6–39.2)	29.7 (22.8–42.4)	0.65
Combined	24.5 (23.4–39.6)	29.1 (23.2–40.5)	0.72
Waist (cm)			
Basal	101 (85–126)	104.5 (78–125)	0.75
Mono	93 (83–125)	99 (75–125)	0.94
Combined	94.5 (85–126)	102.5 (76–120)	0.51

Values are expressed as medians and ranges

Normal ranges for: UFC 90–694 nmol/24 h; LNSC <3.5 ng/ml; ACTH 10–50 pg/ml

Ketoconazole monotherapy

Ketoconazole is an imidazole derivative, an antifungal agent that—at high doses—reduces adrenal steroid production by inhibiting numerous steroidogenic enzymes, e.g. 11-β hydroxylase, 17-hydroxylase and 18-hydroxylase [27, 28]. In some cases, doses between 600–1,200 mg/day are needed to control excessive hormone levels, exposing patients to the risk of adverse effects such as gynecomastia, impaired testicular function, adrenal insufficiency and hepatotoxicity [28, 29]. Castinetti et al. [30] retrospectively studied 38 CD patients treated with ketoconazole, finding it effective in 50 % of cases. In our series, a complete response to ketoconazole was observed in 5/8 patients; there was also an important reduction in UFC levels in the other 3 cases, but the dose had to be reduced in 2 of them because of side effects. The maximal dose of ketoconazole used in this study was quite low; we might have had better results if we had used higher doses, but we preferred to stop at 600 mg/day to avoid side effects and adrenal insufficiency. Compared with cabergoline monotherapy, ketoconazole alone proved more effective in reducing cortisol levels, but the circadian rhythm of cortisol secretion (assessed by measuring LNSC) was not restored in the majority of patients.

Combination therapy

The rationale for using the two drugs in association is to combine their different mechanisms of action to obtain a better hormonal control of the patient's hypercortisolism, administering lower doses of both drugs, causing fewer side effects, and possibly also reducing the treatment escape. To our knowledge, only cabergoline with the subsequent addition of ketoconazole had been studied to date, not the same combination in reverse. In fact, the reduction in cortisol levels initially achievable with by ketoconazole might lead to D2 receptor upregulation in corticotroph tumors, potentially increasing the action of cabergoline on the adenoma, even if it was not proved in vitro [31]. When we compared the

two treatment regimens (cabergoline with added ketoconazole vs ketoconazole with added cabergoline), however, we found no differences between the treatment groups, indicating that it makes no difference which way round the treatment is given: when one drug becomes less effective, the other can be added regardless of the order of their administration. In our series, we obtained an overall UFC normalization in 79 % of patients, which is comparable with the results reported by Vilar et al. [10, 15] Our patients also experienced a clinical improvement while on the combined therapy, in terms of their BMI, waist circumference, hypertension and diabetes control. The only patient (in the group treated initially with ketoconazole) who showed no response at all to cabergoline had a macroadenoma, which reportedly has a low D2 receptor expression [32]. Another observation worth emphasizing is that patients given drugs as their first-line therapy tended to respond less well to medical treatment than those already treated surgically: this may be due to patients with de novo CD having higher hormone levels than those with recurrent or persistent CD. As concerns MRI findings, no differences emerged after the treatment; in particular, patients with no evident adenoma at the baseline did not reveal any lesions after the treatment either. We also measured LNSC as a marker of disease control, comparing the levels in patients on combined therapy with controls in prolonged remission of CD after effective neurosurgery. It is well known that the lack of circadian rhythm in cortisol secretion is one of the typical features of CD, and one of the first markers of its recurrence, before any rise in UFC levels or signs of florid hypercortisolism [33–36]. To our knowledge, only a recent study on medical therapy for CD has considered both UFC and LNSC as a marker of response; the authors observed a recovery of cortisol diurnal rhythm in 50 % of patients with previous impaired cortisol day-night profile [37]. We found that all our controls in remission had normal salivary cortisol levels, whereas this parameter failed to return to normal in most of the patients on medical treatment despite their UFC levels being within the normal range, suggesting an incoming escape phenomenon. In 3 of 4 patients whose LNSC normalized while they were on an extended period of treatment, we observed a gradual rise in this parameter to above the upper limit of normality, followed by a slow increase in UFC levels too, though these remained within the normal range. Persistently high LNSC levels reveal a subclinical hypercortisolism, a sign of inadequate disease control and of the risk of future treatment escape. We also noted that clinical improvements (especially as regards systolic and diastolic blood pressure levels) were more remarkable when a normalization of UFC was accompanied by a reduction in LNSC, whereas the clinical picture improved less when only the former occurred. An improvement in hypertension could depend partly on the relaxing effect of cabergoline on the vascular smooth muscles and a consequently lower peripheral resistance [38]. The major drawback of the present report is the small sample size which could have limited the possibility to find a statistically significant results in presence of a clinically meaningful difference; further studies involving a larger population will be required to confirm our findings, especially the relationship between degree of hypercortisolism and response to treatment. We conclude that combined therapy represents a valid and safe option for controlling hypercortisolism, both the drug combinations tested here working better in the short term than each drug used alone; however the life-long use of such a therapy seems unlikely, given the difficulty of preserving a complete hormonal control in CD and the possibility of treatment escape. In addition, since the circadian rhythm of cortisol secretion was not restored in the majority of our cases, signs and symptoms of the disease may well persist, meaning that a definitive treatment remains the only option for the management of this severe disease.

Ethical standard The experiment complied with current legislation in our country.

Conflict of interest The authors have no conflict of interest to declare.

References

1. Patil CG, Prevedello DM, Lad SP, Vance ML, Thorner MO, Katznelson L, Laws ER Jr (2008) Late recurrences of Cushing's disease after initial successful transsphenoidal surgery. J Clin Endocrinol Metab 93:358–362
2. Atkinson AB, Kennedy A, Wiggam MI, McCance DR, Sheridan B (2005) Long-term remission rates after pituitary surgery for Cushing's disease: the need for long-term surveillance. Clin Endocrinol (Oxf) 63:549–559
3. Rees DA, Hanna FW, Davies JS, Mills RG, Vafidis J, Scanlon MF (2002) Long-term follow-up results of transsphenoidal surgery for Cushing's disease in a single centre using strict criteria for remission. Clin Endocrinol (Oxf) 56:541–551
4. Sheehan JM, Lopes MB, Sheehan JP, Ellegala D, Webb KM, Laws ER Jr (2000) Results of transsphenoidal surgery for Cushing's disease in patients with no histologically confirmed tumor. Neurosurgery 47:33–39
5. Bochicchio D, Losa M, Buchfelder M (1995) Factors influencing the immediate and late outcome of Cushing's disease treated by transsphenoidal surgery: a retrospective study by the European Cushing's Disease Survey Group. J Clin Endocrinol Metab 80:3114–3120
6. Tritos NA, Biller BM, Swearingen B (2011) Management of Cushing disease. Nat Rev Endocrinol 7:279–289
7. Sonino N (1987) The use of ketoconazole as an inhibitor of steroid production. N Engl J Med 317:812–818
8. Feldman D (1986) Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. Endocr Rev 7:409–420
9. Pivonello R, Ferone D, de Herder WW, Kros JM, De Caro ML, Arvigo M, Annunziato L, Lombardi G, Colao A, Hofland LJ, Lamberts SW (2004) Dopamine receptor expression and function

in corticotroph pituitary tumors. J Clin Endocrinol Metab 89: 2452–2462
10. Vilar L, Naves LA, Azevedo MF, Arruda MJ, Arahata CM, Moura E, Silva L, Agra R, Pontes L, Montenegro L, Albuquerque JL, Canadas V (2010) Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease. Pituitary 13:123–129
11. Biller BMK, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J et al (2008) Treatment of ACTH-dependent Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab 93: 2454–2462
12. Hoffman BM, Hlavac M, Martinez R, Buchfelder M, Müller OA, Fahlbusch R (2008) Long-term results after microsurgery for Cushing disease: experience with 426 primary operations over 35 years. J Neurosurg 108:9–18
13. Pivonello R, De Martino MC, Cappabianca P, De Leo M, Faggiano A, Lombardi G, Hofland LJ, Lamberts SW, Colao A (2009) The medical treatment of Cushing's disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in patients unsuccessfully treated by surgery. J Clin Endocrinol Metab 94:223–230
14. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM (2008) The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 93:1526–1540
15. Feelders RA, de Bruin C, Pereira AM, Romijn JA, Netea-Maier RT, Hermus AR, Zelissen PM, van Heerebeek R, de Jong FH, van der Lely AJ, de Herder WW, Hofland LJ, Lamberts SW (2010) Pasireotide alone or with cabergoline and ketoconazole in Cushing's disease. N Engl J Med 362:1846–1848
16. Boscaro M, Ludlam WH, Atkinson B, Glusman JE, Petersenn S, Reincke M, Snyder P, Tabarin A, Biller BM, Findling J, Melmed S, Darby CH, Hu K, Wang Y, Freda PU, Grossman AB, Frohman LA, Bertherat J (2009) Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial. J Clin Endocrinol Metab 94:115–122
17. Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, Schoenherr U, Mills D, Salgado LR, Biller BM, Pasireotide B2305 Study Group (2012) A 12-month phase 3 study of pasireotide in Cushing's disease. N Engl J Med 366:914–924
18. Pecori Giraldi F, Ambrogio AG, Andrioli M, Sanguin F, Karamouzis I, Corsello SM, Scaroni C, Arvat E, Pontercorvi A, Cavagnini F (2012) Potential role for retinoic acid in patients with Cushing's disease. J Clin Endocrinol Metab 97:3577–3583
19. Páez-Pereda M, Kovalovsky D, Hopfner U, Theodoropoulou M, Pagotto U, Uhl E, Losa M, Stalla J, Grübler Y, Missale C, Arzt E, Stalla GK (2001) Retinoic acid prevents experimental Cushing's syndrome. J Clin Invest 108:1123–1131
20. Bevan JS, Webster J, Burke CW, Scanlon MF (1992) Dopamine agonists and pituitary tumor shrinkage. Endocr Rev 13:220–240
21. Mercado-Asis LB, Yasuda K, Murayama M, Mune T, Morita H, Miura K (1992) Beneficial effects of high daily dose bromocriptine treatment in Cushing's disease. Endocrinol Jpn 39: 385–395
22. Lamberts SW, Klijn JG, de Quijada M, Timmermans HA, Uitterlinden P, de Jong FH, Birkenhäger JC (1980) The mechanism of the suppressive action of bromocriptine on adrenocorticotropin secretion in patients with Cushing's disease and Nelson's syndrome. J Clin Endocrinol Metab 51:307–311
23. Colao A, Di Sarno A, Marzullo P, Di Somma C, Cerbone G, Landi ML, Faggiano A, Merola B, Lombardi G (2000) New medical approaches in pituitary adenomas. Horm Res 53:76–87
24. Petrossians P, Thonnard AS, Beckers A (2010) Medical treatment in Cushing's syndrome: dopamine agonists and cabergoline. Neuroendocrinology 92:116–119
25. Godbout A, Manavela M, Danilowicz K, Beauregard H, Bruno OD, Lacroix A (2010) Cabergoline monotherapy in the long-term treatment of Cushing's disease. Eur J Endocrinol 163:709–716
26. Lila AR, Gopal RA, Acharya SV, George J, Sarathi V, Bandgar T, Menon PS, Shah NS (2010) Efficacy of cabergoline in uncured (persistent or recurrent) Cushing disease after pituitary surgical treatment with or without radiotherapy. Endocr Pract 16:968–976
27. Engelhardt D, Weber MM, Miksch T, Abedinpour F, Jaspers C (1991) The influence of ketoconazole on human adrenal steroidogenesis: incubation studies with tissue slices. Clin Endocrinol (Oxf) 35:163–168
28. Sonino N, Boscaro M (1999) Medical therapy for Cushing's disease. Endocrinol Metab Clin North Am 28:211–222
29. Sonino N, Boscaro M, Paoletta A, Mantero F, Ziliotto D (1991) Ketoconazole treatment in Cushing's syndrome: experience in 34 patients. Clin Endocrinol (Oxf) 35:347–352
30. Castinetti F, Morange I, Jaquet P, Conte-Devolx B, Brue T (2008) Ketoconazole revisited: a preoperative or postoperative treatment in Cushing's disease. Eur J Endocrinol 158:91–99
31. de Bruin C, Feelders RA, Waaijers AM, van Koetsveld PM, Sprij-Mooij DM, Lamberts SW, Hofland LJ (2009) Differential regulation of human dopamine D2 and somatostatin receptor subtype expression by glucocorticoids in vitro. J Mol Endocrinol 42:47–56
32. de Bruin C, Pereira AM, Feelders RA, Romijn JA, Roelfsema F, Sprij-Mooij DM, van Aken MO, van der Lelij AJ, de Herder WW, Lamberts SW, Hofland LJ (2009) Coexpression of dopamine and somatostatin receptor subtypes in corticotroph adenomas. J Clin Endocrinol Metab 94:1118–1124
33. Carrasco CA, Coste J, Guignat L, Groussin L, Dugué MA, Gaillard S, Bertagna X, Bertherat J (2008) Midnight salivary cortisol determination for assessing the outcome of transsphenoidal surgery in Cushing's disease. J Clin Endocrinol Metab 93: 4728–4734
34. Raff H (2009) Utility of salivary cortisol measurements in Cushing's syndrome and adrenal insufficiency. J Clin Endocrinol Metab 94:3647–3655
35. Nunes ML, Vattaut S, Corcuff JB, Rault A, Loiseau H, Gatta B, Valli N, Letenneur L, Tabarin A (2009) Late-night salivary cortisol for diagnosis of overt and subclinical Cushing's syndrome in hospitalized and ambulatory patients. J Clin Endocrinol Metab 94:456–462
36. Bou Khalil R, Baudry C, Guignat L, Carrasco C, Guibourdenche J, Gaillard S, Bertagna X, Bertherat J (2011) Sequential hormonal changes in 21 patients with recurrent Cushing's disease after successful pituitary surgery. Eur J Endocrinol 165:729–737
37. van der Pas R, de Bruin C, Pereira AM, Romijn JA, Netea-Maier RT, Hermus AR, Zelissen PM, de Jong FH, van der Lely AJ, de Herder WW, Webb SM, Lamberts SW, Hofland LJ, Feelders RA (2012) Cortisol diurnal rhythm and quality of life after successful medical treatment of Cushing's disease. Pituitary doi. doi: 10.1007/211102-012-0452-2
38. Murphy MB (2000) Dopamine: a role in the pathogenesis and treatment of hypertension. J Hum Hypertens 14:S47–S50