CLINICAL STUDY

Efficiency and tolerance of mitotane in Cushing's disease in 76 patients from a single center

Camille Baudry^{1,2,6,7}, Joël Coste^{2,7}, Roula Bou Khalil¹, Stéphane Silvera^{5,7}, Laurence Guignat^{1,7}, Jean Guibourdenche⁴, Halim Abbas³, Paul Legmann^{5,7}, Xavier Bertagna^{1,6,7} and Jérôme Bertherat^{1,6,7}

¹Department of Endocrinology, Reference Center for Rare Adrenal Diseases, Assistance Publique-Hôpitaux de Paris, Cochin Hospital, 27 Rue du Faubourg Saint-Jacques, 75014 Paris, France, ²Biostatistics Unit APEMAC, EA 4360, Nancy-Université, Nancy 54000, France, Departments of ³Toxicology, ⁴Biophysics and Hormonology and ⁵Radiology, Assistance Publique-Hôpitaux de Paris, Cochin Hospital, 27 Rue du Faubourg Saint-Jacques, 75014 Paris, France, ⁶Institut National de la Santé et de la Recherche Médicale Unité 1016, CNRS UMR8104, Institut Cochin, Paris, France and ⁷Faculté de Médecine, Université Paris Descartes, 75005 Paris, France

(Correspondence should be addressed to J Bertherat; Email: jerome.bertherat@cch.aphp.fr)

Abstract

Context: Alternatives to transsphenoidal pituitary surgery may be required in Cushing's disease (CD) as a first- or second-line treatment. Mitotane is a potent anti-cortisolic drug but has been rarely investigated in the treatment of CD.

Objective: Evaluation of the efficacy and tolerance of mitotane in CD patients.

Design and setting: Retrospective analysis of 76 patients treated with mitotane from 219 patients diagnosed with CD between 1993 and 2009 in a single center.

Main outcome measure: Remission was defined as normalization of 24-h urinary free cortisol (24-h-UFC).

Results: Remission was achieved in 48 (72%) of the 67 long-term treated patients, after a median time of 6.7 (5.2–8.2) months. Mean plasma mitotane concentration at the time of remission was 10.5 ± 8.9 mg/l, with a mean daily dose of 2.6 ± 1.1 g. A negative linear relationship was observed between plasma mitotane concentration and 24-h-UFC ($P < 0.0001$). Seventeen of 24 (71%) patients with durable remission subsequently experienced recurrence, after a median time of 13.2 (5.0–67.9) months. At the time of treatment discontinuation, ACTH concentration was statistically associated with a lower recurrence probability (hazard ratios 0.57 (0.32–1.00), $P = 0.05$). Intolerance leading to treatment discontinuation occurred in 19 patients (29%). A pituitary adenoma became identifiable during mitotane treatment in 12 (25%) of the 48 patients with initial negative pituitary imaging allowing subsequent transsphenoidal surgery.

Conclusion: Mitotane is useful at different stages of CD. Mitotane dose adjustment based on plasma concentration monitoring and side effects could control hypercortisolism in the majority of CD patients.

European Journal of Endocrinology 167 473–481

Introduction

Cushing's syndrome is associated with a high morbidity and can be lethal when not treated. Its most frequent cause is Cushing's disease (CD). The first-line treatment for CD is transsphenoidal surgery (TSS) that selectively removes the causative corticotroph adenoma (mostly microadenomas) (1), but despite modern imaging by magnetic resonance imaging (MRI), a pituitary tumor is not visible in 35–65% of patients (2). Immediate disease remission is achieved in about 60–85% of patients after pituitary surgery (3, 4) but late recurrence occurs in 5–20% of patients (5).

Alternative therapeutic approaches are therefore needed for CD, either as first-line treatment, after failure of pituitary surgery, or in the case of CD recurrence. Pituitary irradiation is one alternative but, despite substantial progress in radiotherapeutic methods, efficacy

is not immediate and there are significant side effects (6). Bilateral adrenalectomy immediately controls excess cortisol but requires lifelong steroid-substitution therapy. Moreover, after bilateral adrenalectomy, the corticotroph pituitary tumor may progress, requiring additional targeted pituitary treatment (7). The main pharmacotherapeutic treatments for CD are adrenal-blocking drugs such as metyrapone, ketoconazole, and 1,ortho-1, para'-dichloro-diphényl-dichloro-ethane (o,p'DDD), also termed mitotane (3, 8, 9, 10). Other drugs that directly target ACTH secretion have recently been developed but induce only limited and/or transient remission (11, 12, 13, 14) of CD.

The anti-cortisolic effect of mitotane was first described in dogs and subsequently in humans in 1961 (15, 16). In addition to enzyme-inhibitory activity against 11 β -hydroxylation and cholesterol side chain cleavage, mitotane has a strong adrenolytic effect that

confers long-lasting activity and could limit the escape phenomenon observed with other anti-cortisolic drugs (17). There is now evidence for the anti-proliferative and anti-secretory effects of mitotane in adrenocortical cancer (18, 19, 20), but there are no recent data on its long-term efficacy or safety in the treatment of CD. The largest study to date was published from our center in 1979 and included 62 patients treated with mitotane, of whom 24 also received pituitary irradiation (21). The aim of the current study was to analyze the efficacy and side effects of mitotane as a first- or second-line treatment in patients with CD in a single center.

Materials and methods

Patients

A total of 76 consecutive patients in the endocrinology department of Cochin Hospital diagnosed with CD between January 1993 and December 2009 and treated with mitotane were analyzed retrospectively. These patients are part of a cohort of 248 patients with ACTH-dependant Cushing's syndrome followed during the same period. This population was different from that previously studied in our center (21). We excluded patients with proven or suspected ectopic ACTH secretion (29 patients) and patients with mitotane treatment initiated outside our center and for whom no data were available for the treatment period (ten patients). Of the 76 patients studied, none had previously received radiotherapy, except one patient who was treated with mitotane for persisting CD 3 years after gamma knife radiosurgery.

Before 2004, mitotane was administered orally with 500 mg capsules (mitotane APHP) provided by the Assistance Publique-Hôpitaux de Paris pharmacy. From 2004 onward, oral mitotane was administered in the form of 500 mg Lysodren tablets (HRA Pharma, Paris, France). These two mitotane formulations have different absorption rates: three 500 mg tablets of the APHP formulation are considered equivalent to one 500 mg Lysodren tablet (22). Thus, for clarity, the Lysodren-equivalent dose is used in this study. For doses up to 4 g/day, mitotane was administered in three daily doses and gradually tapered to the minimal dose required to maintain remission. The majority of patients began their treatment in hospital for close monitoring and were reevaluated almost every 3 months during the first year of treatment. Since the aim was to block and then substitute, almost all patients received concomitant hydrocortisone replacement except for those with persisting hypercortisolism. The study was performed according to the French rules for noninterventional monocentric clinical record analysis with approval by the national committee for clinical computed database management (CNIL).

Diagnosis of CD

ACTH-dependent Cushing's syndrome was diagnosed on the basis of classical clinical findings and laboratory criteria (23), including urinary free cortisol (UFC) excretion above 90 µg/24 h (248 nmol/24 h), midnight plasma cortisol above 75 ng/ml (207 nmol/l) or midnight salivary cortisol above 2 ng/ml (5.52 nmol/l), and/or plasma cortisol after overnight 1 mg dexamethasone suppression test above 18 ng/ml (50 nmol/l), and unsuppressed plasma ACTH.

The pituitary origin of ACTH was determined with various levels of certainty. 'Proven' CD (56 patients) was defined by histological confirmation of corticotroph adenoma and/or postsurgical corticotroph deficiency and/or central-to-peripheral gradient in bilateral inferior petrosal sinus venous sample. 'Highly suspected' CD (20 patients) was defined by the absence of the preceding criteria but the presence of at least one dynamic test result consistent with CD among corticotropin-releasing hormone stimulation test, metyrapone stimulation test, or high-dose (8 mg) dexamethasone suppression test, along with no evidence for an ectopic source of ACTH during the follow-up.

Endocrine follow-up, remission, and recurrence criteria

Clinical and hormonal data were analyzed before mitotane treatment, 3–6 months after the beginning of treatment, and then yearly. 24-h-UFC was assayed off-hydrocortisone substitutive therapy with medical surveillance. Remission at follow-up was defined as either normalization of 24-h-UFC (<90 µg) or hypocortisolism. Hypocortisolism was defined as a morning plasma cortisol concentration <50 ng/ml (138 nmol/l) or <210 ng/ml (580 nmol/l) after 250 µg ACTH-stimulation test using Synacthen. Recurrence of hypercortisolism after treatment withdrawal was defined as an elevated 24-h-UFC (>90 µg), with Cushing's syndrome's clinical symptoms, and the need for a new therapeutic intervention.

Biological assays and imaging

Cortisol and ACTH were assayed as previously reported (24, 25). The mitotane plasma concentration assay was used routinely in our center from the year 2000 and was measured using an isocratic HPLC. Samples were deproteinised with cold acetone, and mitotane concentrations were quantified using a diode-array detector with 1,1-dichloro,2,2-bis (P-chlorophenyl) ethylene as an internal standard (26).

All patients underwent pituitary MRI before mitotane treatment and had regular MRIs during the follow-up. Pituitary MRI scans were performed with coronal and sagittal T1 weighting, with and without enhancement, and with coronal T2 weighting. When the initial MRI

was negative but the MRI during follow-up had revealed an adenoma or if the tumor size increased during treatment, we performed a second reading of MRIs: a single radiologist analyzed at the same time, retrospectively and without clinical information on outcome and current treatment of the patients concerned, all pituitary MRIs in chronological order for each patient. The results were compared with those obtained at the time at which the MRI scans were actually performed.

Statistical analysis

Data are reported using median and range, or percentages as appropriate. Given the non-normal distribution of several variables and the small numbers of subjects in some groups of interest, we used nonparametric statistical methods to study the relationship between variables when appropriate (Fisher's exact test, Wilcoxon test, and pairwise Wilcoxon test). We used two-tailed tests, and P values <0.05 were considered significant. Remission, recurrence, and time-to-event were estimated by the Kaplan–Meier method, with follow-up starting at the beginning of mitotane treatment for remission. At the time of treatment withdrawal, the patient was censored. For recurrence, follow-up started at the end of the treatment in patients achieving remission. The predictive values of clinical and biological criteria for remission, intolerance, and relapse were evaluated using Cox's proportional hazards regression models, which were used to estimate crude and adjusted hazard ratios (HRs) and their 95% confidence intervals (95% CIs). The relationships between 24-h-UFC and mitotane current dose or mitotane plasma concentration were assessed using linear mixed models adapted for repeated measures (27). The dependent variable (24-h-UFC) was log-transformed as residuals departed from normality. Statistical analysis was performed using SAS version 9.2 (SAS institute, Inc., Cary, NC, USA).

Results

A total of 76 patients were treated with mitotane for CD diagnosed between January 1993 and December 2009 in Cochin Hospital. The main characteristics of these patients are described in Table 1. Among them, 49 patients were treated with mitotane as a first-line treatment, while 27 as second-line treatment, i.e. after TSS.

Remission and its determinants

Nine patients received mitotane very transiently, as preparation for planned pituitary surgery was temporarily delayed due to severe hypercortisolism. These patients were excluded from the efficacy analysis. Among the 67 other patients, remission was obtained in 48 (72%) patients after a median time of 6.7 months

Table 1 Main characteristics of the 76 patients treated with mitotane, either as a first-line treatment or as a second-line therapy after pituitary surgery. Data are expressed as number (percentage) or median (range). The mean daily dose was calculated by dividing the cumulative dose by treatment duration in days.

	First-line treatment (n=49)	Second-line treatment (n=27)
Baseline characteristics		
Gender: women/men	36 (73)/13 (27)	23 (85)/4 (15)
Age at diagnostic (years)	39 (14–71)	34 ± 12 (14–61)*
Baseline hormoneology		
Urinary cortisol (µg/24 h)	383 (84–3750)	240 (122–1094)*
Plasma ACTH (pg/ml)	65 (16–2100)	51 (20–216)*
Negative baseline MR imaging	39 (80)	15 (56)*
Indication		
No pituitary adenoma	39 (80)	–
Invasive pituitary adenoma	3 (6)	–
Pituitary surgery failure	–	16 (59)
Relapse after pituitary surgery	–	9 (33)
Preparation before surgery	7 (14)	2 (8)
Treatment characteristics		
Initial daily dose (g/day)	2.7 (0.3–4.5)	2.0 (1.0–8.0)
Treatment duration (months)	6.9 (0.3–114.9)	16.4 (0.8–68.9)
Cumulative dose (g)	543 (43–4435)	1076 (46–5676)*
Mean daily dose (g/day)	2.5 (1.1–4.3)	2.4 (0.9–6.1)
Bilateral adrenalectomy during follow-up	19 (39)	10 (37)
Pituitary radiotherapy during follow-up	4 (8)	5 (19)

MR, magnetic resonance. * $P < 0.05$ compared with first-line treatment group.

(95% CIs 5.2–8.2; Fig. 1). Of the 19 patients who did not achieve remission, treatment was withdrawn due to lack of efficacy in ten patients (after a median period of 7.9 months) and due to intolerance in nine patients (after a median period of 2.2 months). Of note, in the ten patients who discontinued treatment due to lack of efficacy, there was a mean decrease in 24-h-UFC of 50%.

Univariate analysis was performed to identify predictive factors of remission and no significant difference was observed between the mitotane first- and second-line treatment groups. Similarly, patient age, gender, weight or BMI, initial mitotane dose, severity of baseline hypercortisolism, and baseline plasma ACTH concentration were not significant predictors of response to therapy.

Clinical and hormonal course with mitotane

The main hormonal and metabolic parameters, before and during mitotane treatment, in the patients who experienced remission are shown in Table 2. Mean ACTH levels increased from 67.8 ± 65.8 to 221.3 ± 180.9 pg/ml ($P < 0.0001$). There was a statistically significant improvement in all metabolic parameters after 6 months of treatment, except in systolic blood pressure and lipid profile. Total cholesterol increased

Figure 1 Kaplan–Meier estimation: probability of remission according to time after mitotane treatment was started.

due to increases in LDL-cholesterol from 3.5 ± 1.1 to 4.7 ± 2.2 mmol/l ($P < 0.05$) and HDL-cholesterol from 1.6 ± 0.5 to 2.0 ± 0.7 mmol/l ($P < 0.05$). However, the LDL/HDL-cholesterol ratio remained stable, from 2.4 ± 1.4 to 2.4 ± 1.5 ($P = 0.43$). Triglycerides were also slightly increased from 1.7 ± 1.8 to 1.9 ± 1.1 mmol/l ($P < 0.01$). Of note, seven patients were being treated with statins at the start of mitotane treatment and 13 patients commenced statin therapy during the treatment.

Relationship between plasma mitotane concentrations and remission

Plasma mitotane was routinely measured in 36 patients, representing a total of 108 distinct measures. At the time of remission, mean plasma mitotane concentration in the remission group was 10.6 ± 5.2 mg/l ($n = 24$), with a mean daily dose of 2.7 ± 1.2 g/day.

There was no statistically significant relationship between plasma mitotane concentration and current

daily dose at any time-point. Moreover, no statistically significant relationship was found between 24-h-UFC and concomitant daily dose of mitotane.

However, a negative linear relationship was observed between plasma mitotane concentration and 24-h-UFC; an increase of 1 mg/l plasma mitotane concentration was associated with a decrease of 10.4 µg for 24-h-UFC. Owing to a mild departure from normality in the residuals, 24-h-UFC was log-transformed. This transformation resulted in a highly significant negative linear relationship of 24-h-UFC with plasma mitotane concentrations ($P < 0.0001$). There were 73 concomitant measures of mitotane plasma concentration and 24-h-UFC. Patients with a mitotane plasma concentration above 8.5 mg/l did not have abnormal concomitant 24-h-UFC values (Fig. 2).

Adverse effects and clinical tolerance to mitotane

Adverse effects that occurred during mitotane treatment were divided into two groups: serious intolerance that led to treatment discontinuation and mild intolerance where treatment was continued. The most frequent adverse effects were gastrointestinal (47% of patients) and neurologic intolerance (30% of patients; Table 3). The retrospective nature of the study did not allow investigation of whether these adverse events were treatment related.

A total of 19 patients showed serious adverse effects leading to treatment withdrawal, accounting for 28% of the 67 patients who received mitotane not only for preparation of surgery. Of these 19 patients, ten had already reached remission at the time of discontinuation. Univariate analysis showed that none of the baseline characteristics studied (first- or second-line treatment, age, gender, weight, BMI, starting dose of mitotane, mitotane formulation: mitotane APHP or Lysodren, and severity of hypercortisolism)

Table 2 Comparison of hormonal parameters and metabolic outcomes in the 45 patients in whom remission had been obtained, with available data, before and 6 months after mitotane initiation. Normal ranges for urinary cortisol, 5–90 µg/24 h and plasma ACTH, 5–60 pg/ml. Data are expressed as median (range).

Parameter	Before mitotane	Under mitotane	P value
Urinary cortisol (µg/24 h)	285.5 (104–2457)	44 (7–182)	<0.0001
Plasma ACTH (pg/ml)	43 (16.6–2100)	184 (21–149)	<0.0001
BMI (kg/m ²)	28.3 (19.3–51.7)	26.2 (16.3–46.3)	<0.0001
Systolic blood pressure (mmHg)	130 (110–190)	125 (100–160)	0.22
Diastolic blood pressure (mmHg)	80 (50–120)	70 (50–100)	<0.01
Fasting serum glucose (mmol/l)	4.9 (3.9–14.9)	4.3 (2.6–7.2)	<0.01
Post prandial serum glucose (mmol/l)	7.5 (4.6–15.2)	6.3 (5–10.5)	<0.01
Total cholesterol (mmol/l)	5.8 (3.5–8.2)	7.7 (5.1–14)	<0.0001
LDL-cholesterol (mmol/l)	3.7 (1.4–5.7)	4.2 (1.9–10.9)	<0.05
HDL-cholesterol (mmol/l)	1.6 (0.8–3.1)	1.8 (1.0–3.6)	<0.05
Triglycerides (mmol/l)	1.2 (0.4–11.8)	1.6 (0.6–5.5)	<0.01

Figure 2 Relationship between plasma mitotane concentration ($\mu\text{g/ml}$) and 24-h-UFC (μg). Urinary cortisol is represented with a logarithmic scale. The horizontal dotted line corresponds to 90 μg (upper limit of normal range of 24-h-UFC). Filled circles correspond to 73 distinct concomitant measures of 24h-UFC and plasma mitotane level in 36 different patients. The vertical dotted line corresponds to the mitotane plasma level above which no concomitant 24-h-UFC is above the normal range.

were statistically associated with the occurrence of serious intolerance.

Only two patients had a mitotane plasma concentration above 20 mg/l (22 and 25 mg/l) during the course of treatment. In one of these patients, this was associated with signs of neurotoxicity.

Characterization of hypercortisolism recurrence after mitotane discontinuation

Among the 48 patients who achieved remission, four patients were still being treated with mitotane at the time of this analysis. Treatment was discontinued in the remaining 44 patients for different reasons. In 18 patients, another treatment was immediately performed: in this group of patients, mitotane was discontinued because of pituitary adenoma visualization during the imaging follow-up allowing TSS in eight of them, relapse of hypercortisolism under mitotane treatment in five of them (for these five patients, the mitotane daily dose had to be reduced after having achieved initial remission because of poor clinical tolerance at higher doses), and other reasons like patient's choice or, in five of them, moving to a country without mitotane available.

The other patients ($n=26$) had discontinued treatment without an alternative treatment because of controlled disease ($n=13$), serious intolerance ($n=9$), and pregnancy wish ($n=4$). Among these patients, 24 were at risk of recurrence, i.e. in remission, at the time of mitotane discontinuation, without another therapeutic intervention, and not lost to follow-up. At the time of mitotane discontinuation, 18 of these 24 patients (75%) presented adrenal insufficiency. Median follow-up duration after treatment discontinuation was 71 months (29–126). During this follow-up,

17 (71%) patients showed recurrence of hypercortisolism. The median time-to-recurrence after mitotane discontinuation was 13.2 months (5.0–67.9, 95% confidence limits; Fig. 3).

Univariate analysis showed that recurrence was not statistically associated with age, gender, last dose of mitotane, last plasma mitotane concentration, total treatment duration, or cumulative dose of mitotane. Adrenal insufficiency at the time of treatment withdrawal was not statistically predictive of recurrence nor were 24-h-UFC or morning plasma cortisol concentrations. Conversely, a higher ACTH concentration at the time of treatment withdrawal was significantly associated with a lower probability of recurrence (HR 0.57 (0.32–1.00), $P=0.05$), but this relation was not a linear one. At the time of treatment withdrawal, compared with patients with plasma ACTH ≤ 114 pg/ml (the first quartile of the 24 patients at risk of recurrence), patients with plasma ACTH > 114 pg/ml had an almost statistically significant lower probability of recurrence (HR 0.36 (0.13–1.02), $P=0.05$).

Evolution of pituitary imaging during mitotane treatment

Despite a negative MRI before treatment, in 12 patients, a pituitary adenoma became apparent upon MRI during or at the end of mitotane treatment ('emergent pituitary adenoma'), after a median time of 10.9 (range: 3.8–94.6) months. Ten of these 12 patients were treated with mitotane as first-line treatment, accounting for 25.6% of the 39 patients with no initial adenoma visualization and mitotane as first-line treatment. For the remaining two

Table 3 Main adverse effects observed during mitotane therapy in the 76 patients who received the treatment. Gastrointestinal signs included anorexia, nausea/vomiting, and diarrhea. Neurologic signs included dysarthria, ataxia, confusion, dizziness, and paresthesia. Mild neutropenia defined as a neutrophil count between 1000 and 1500/mm³. Other side effects: one patient developed hemolysis and hemostasis abnormality and one patient developed acute depression.

	Mild intolerance	Serious intolerance
Gastrointestinal signs	36 (47.4%)	5 (6.6%)
Increased transaminases	13 (17.1%)	1 (1.3%)
> ULN	11	
> 3 $\times$ ULN	2	
Increased GGT	36 (47.4%)	
> 3 $\times$ ULN	24	
> 5 $\times$ ULN	12	
Neurologic signs	23 (30.3%)	6 (9%)
Lipid disorders	54 (71.1%)	
LDL-cholesterol (> 3.35 mmol/l)	15 (19.7%)	
LDL-cholesterol (> 5.16 mmol/l)	19 (25%)	
Triglycerides (> 2.28 mmol/l)	25 (32.9%)	
Mild neutropenia	5 (6.6%)	
Skin rash	3 (3.9%)	5 (6.6%)
Gynecomastia (men)	3 (17.6%)	
Other		2 (2.6%)

ULN, upper limit of normal range.

Figure 3 Kaplan–Meier estimation of recurrence in the 24 ‘at-risk’ patients after mitotane was discontinued.

of the 12 patients with an emergent pituitary adenoma, mitotane was administered as second-line treatment after TSS failure, accounting for 2.2% of the nine patients with negative initial MRI and TSS failure.

Of the 12 patients with an emergent pituitary adenoma, seven were in remission at the time of adenoma detection, two had recurrence, and three had noncontrolled disease. Of them, ten patients had TSS leading to immediate remission in all these cases.

Discussion

This study provides an extended view of the efficacy and tolerance of mitotane therapy in CD patients and its potential in CD management as a first-line treatment or as second-line treatment after pituitary surgery failure. Among the 76 patients of this study, nine received a short course of mitotane treatment, which allowed partial control of severe hypercortisolism before a delayed TSS. For the remaining patients, complete remission with mitotane was achieved for 48 (72%) patients, within a median time of almost 6 months. These findings correlate with the pharmacokinetic characteristics of the drug. Mitotane is a lipophilic molecule, stored in adipose tissue resulting in a prolonged half-life and a delayed onset of action. A negative linear relationship between plasma mitotane concentrations and 24-h-UFC was observed. Conversely, there was no relationship between current mitotane dose and plasma mitotane concentration or hormonal control. These results are consistent with pharmacological observations of mitotane in adrenocortical cancer (28, 29, 30). Our study highlights that monitoring of plasma mitotane concentrations is of particular interest in controlling hypercortisolism. The narrow therapeutic range of mitotane is well known, and a target plasma concentration between 14 and 20 mg/l is recommended in adrenocortical cancer (31, 32, 33). However, the target concentration for

the anti-cortisol effect is not well established. In our study, plasma mitotane concentrations ≥ 8.5 mg/l were associated with normal 24-h-UFC at all time-points during patient follow-up. The previously suggested therapeutic range of mitotane for control of Cushing’s syndrome (34) appears to be lower than that for the antitumoral effect. This could improve tolerance, as a lower dose of mitotane would be needed to reach hormonal control in CD. Furthermore, after achieving control of cortisol secretion, mitotane dose could be progressively tapered off. Based on this experience, we would recommend to start mitotane at rapidly increasing dose during the first 4–6 weeks, up to 2–4 g/day depending on the patient profile and the urinary cortisol levels. Initially, a monthly assay of plasma mitotane is helpful for dose adjustment. Except for patients with very high urinary cortisol, substitutive therapy has to be gradually introduced after 2–3 weeks in outpatients. Patient education about adrenal insufficiency is important in all cases. When monitoring plasma cortisol, one should keep in mind that cortisol binding globulin is increased by mitotane, leading to increased plasma cortisol levels. However, salivary and urinary cortisols are not affected by this phenomenon.

We did not identify any predictive factors of remission among age, weight, or BMI of the patients. There was also no association between the probability of remission and current disease status (severity of hypercortisolism and treatment indication: first- or second-line treatment) or treatment starting dose. Remission of CD was associated with an improvement in all metabolic parameters, except LDL-cholesterol. However, the LDL/HDL ratio did not worsen.

These results are consistent with the majority of other published findings. Indeed, the largest studies published 30 years ago (21, 35) reported a remission rate of 80%, but in association with pituitary irradiation in most cases, and with less precise remission criteria than the criteria used in our study. These data are obviously no longer relevant in assessing mitotane efficacy for CD management, as modern pituitary imaging and pituitary surgery emerged after these studies. More recent studies have been relatively small, including less than ten patients (36).

Although TSS is currently the only curative treatment for CD, it is often difficult to perform due to the lack of pituitary adenoma visualization in 35–65% of patients. Invasive pituitary adenomas are not accessible for complete resection. Moreover, immediate and long-term remission are not always achieved with TSS. In a recent study, the recurrence rate of CD after TSS due to microadenoma was 25%, with a median time to recurrence of 39 months (4). In these cases, a second TSS is possible, but the immediate remission rate is lower (37, 38).

There is therefore a need for alternative or complementary therapeutic approaches. Other studies have reported various remission rates with medical

treatments: 75% remission with short-term metyrapone (8), 51% with ketoconazole (10, 39) and 25–35% of 24-h-UFC normalization with the pituitary-targeted agent cabergoline (11, 40). In very severe forms of Cushing's syndrome, combination therapy with metopirone, ketoconazole, and mitotane have been used successfully (41). Another pharmacologic agent, pasireotide, has been investigated in a phase III randomized double-blind trial comparing two doses in CD (14). Preliminary results showed a limited remission rate of only 29% with pasireotide monotherapy, with disturbance of glucose homeostasis as the main adverse event (13). With these different pharmacological approaches, a combination of several medications often seems necessary to adequately control CD (13, 40). In our study, complete remission was achieved in 48 patients with mitotane monotherapy.

Other second-line therapeutic options for CD are radiotherapy or radiosurgery, but these options have delayed efficacy and remission rates of about 50%. Finally, the ultimate option for CD treatment is bilateral adrenalectomy, leading to definitive adrenal deficiency requiring lifetime steroid replacement therapy.

Owing to the long follow-up period (mean time 97 months, range 6.3–192 months), we were able to assess the long-term management of CD patients treated with mitotane. After remission, some patients subsequently underwent TSS. In the subgroup of patients that were in remission, and whose mitotane treatment had been discontinued without further therapeutic intervention, the overall recurrence rate was 71%. Seven out of 24 patients (29%) did not experience a recurrence of hypercortisolism, despite treatment discontinuation. Indeed, unlike steroidogenesis inhibitors, mitotane is also cytotoxic for the adrenocortical cells. This might explain, in a subset of patients, a long lasting effect due to this adrenolytic effect. Only a high plasma ACTH concentration at the time of treatment withdrawal seems to be associated with a lower probability of recurrence. We hypothesize that a higher ACTH concentration is a reflection of the extent of adrenal suppression. This might be due to increased tumoral ACTH secretion secondary to reduced cortisol feedback on the tumor cells and, to a lesser extent, reactivation of the normal corticotroph cells with cortisol excess correction.

During follow-up, at least 25% of patients with a negative initial MRI had later MRI visualization of a pituitary adenoma after mitotane treatment. This is particularly interesting as mitotane treatment could allow control of cortisol secretion until better visualization of the causal pituitary tumor is possible.

Regarding the tolerance of mitotane, we observed frequent mild side effects, mainly affecting the gastrointestinal and nervous systems, as previously reported (42). However, these adverse effects could be managed by tapering the mitotane dose and/or with additional symptomatic treatment in nearly three-quarters of

cases. In some cases, these side effects could have been related to insufficient steroid replacement in parallel with mitotane treatment. Mild elevation of γ -glutamyltransferase levels was quite frequent, but significant transaminase elevation (above three times the upper limit of normal range) was only noticed in three patients. No cases of hepatocellular deficiency occurred. Finally, mitotane was associated with a mild increase in LDL- and HDL-cholesterol as previously reported (42, 43). This has been attributed to the inhibition of cytochrome P450 (44).

Of the 76 patients studied, six women underwent therapeutic abortion due to pregnancy occurring under treatment or immediately after mitotane discontinuation. Four women had normal pregnancies after treatment discontinuation and a sufficient latency period, resulting in healthy babies with no identifiable malformities.

In conclusion, this retrospective study of a large cohort of CD patients highlights the efficacy and tolerance of mitotane therapy in this rare disease. Treatment with mitotane is useful in the management of different stages of the disease, either as a first-line therapy when pituitary surgery is not appropriate or because of the severity of hypercortisolism, or as a second-line therapy after TSS failure or recurrence of CD. The hormonal control of CD with mitotane therapy is highly associated with the mitotane plasma concentration and its monitoring would help to reach efficacy and manage tolerance.

Declaration of interest

J Bertherat has been involved as a clinical investigator in trials in Cushing's syndrome sponsored by Novartis.

Funding

The management of the database used for this analysis was supported in part by Novartis.

Acknowledgements

The authors thank the staff of the Endocrinology Department of Cochin Hospital for managing patients and the staff of the Hormonal Biology Department of Cochin Hospital for hormone assays. The management of the database used for this analysis was supported in part by Novartis.

References

- 1 Newell-Price J, Bertagna X, Grossman AB & Nieman LK. Cushing's syndrome. *Lancet* 2006 **367** 1605–1617. (doi:10.1016/S0140-6736(06)68699-6)
- 2 Hall WA, Luciano MG, Doppman JL, Patronas NJ & Oldfield EH. Pituitary magnetic resonance imaging in normal human volunteers: occult adenomas in the general population. *Annals of Internal Medicine* 1994 **120** 817–820.
- 3 Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ,

- Klibanski A, Lacroix A, Lindsay JR, Newell-Price J, Nieman LK, Petersenn S, Sonino N, Stalla GK, Swearingen B, Vance ML, Wass JA & Boscaro M. Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2454–2462. (doi:10.1210/jc.2007-2734)
- 4 Patil CG, Prevedello DM, Lad SP, Vance ML, Thorner MO, Katznelson L & Laws ER Jr. Late recurrences of Cushing's disease after initial successful transsphenoidal surgery. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 358–362. (doi:10.1210/jc.2007-2013)
- 5 Atkinson AB, Kennedy A, Wiggam MI, McCance DR & Sheridan B. Long-term remission rates after pituitary surgery for Cushing's disease: the need for long-term surveillance. *Clinical Endocrinology* 2005 **63** 549–559. (doi:10.1111/j.1365-2265.2005.02380.x)
- 6 Castinetti F, Nagai M, Morange I, Dufour H, Caron P, Chanson P, Cortet-Rudelli C, Kuhn JM, Conte-Devolx B, Regis J & Brue T. Long-term results of stereotactic radiosurgery in secretory pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 3400–3407. (doi:10.1210/jc.2008-2772)
- 7 Assie G, Bahurel H, Coste J, Silvera S, Kujas M, Dugue MA, Karray F, Dousset B, Bertherat J, Legmann P & Bertagna X. Corticotroph tumor progression after adrenalectomy in Cushing's disease: a reappraisal of Nelson's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 172–179. (doi:10.1210/jc.2006-1328)
- 8 Verhelst JA, Trainer PJ, Howlett TA, Perry L, Rees LH, Grossman AB, Wass JA & Besser GM. Short and long-term responses to metyrapone in the medical management of 91 patients with Cushing's syndrome. *Clinical Endocrinology* 1991 **35** 169–178. (doi:10.1111/j.1365-2265.1991.tb03517.x)
- 9 Nieman LK. Medical therapy of Cushing's disease. *Pituitary* 2002 **5** 77–82. (doi:10.1023/A:1022308429992)
- 10 Castinetti F, Morange I, Jaquet P, Conte-Devolx B & Brue T. Ketoconazole revisited: a preoperative or postoperative treatment in Cushing's disease. *European Journal of Endocrinology* 2008 **158** 91–99. (doi:10.1530/EJE-07-0514)
- 11 Pivonello R, De Martino MC, Cappabianca P, De Leo M, Faggiano A, Lombardi G, Hofland LJ, Lamberts SW & Colao A. The medical treatment of Cushing's disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in patients unsuccessfully treated by surgery. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 223–230. (doi:10.1210/jc.2008-1533)
- 12 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. Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 115–122. (doi:10.1210/jc.2008-1008)
- 13 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. Pasireotide alone or with cabergoline and ketoconazole in Cushing's disease. *New England Journal of Medicine* 2010 **362** 1846–1848. (doi:10.1056/NEJMc1000094)
- 14 Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, Schoenherr U, Mills D, Salgado LR & Biller BM. A 12-month phase 3 study of pasireotide in Cushing's disease. *New England Journal of Medicine* 2012 **366** 914–924. (doi:10.1056/NEJMoA1105743)
- 15 Wallace EZ, Silverstein JN, Villadolid LS & Weisenfeld S. Cushing's syndrome due to adrenocortical hyperplasia. Treatment with an inhibitor of adrenocortical secretions. *New England Journal of Medicine* 1961 **265** 1088–1093. (doi:10.1056/NEJM196111302652204)
- 16 Southren AL, Tochimoto S, Strom L, Ratuschni A, Ross H & Gordon G. Remission in Cushing's syndrome with o,p'-DDD. *Journal of Clinical Endocrinology and Metabolism* 1966 **26** 268–278. (doi:10.1210/jcem-26-3-268)
- 17 Temple TE Jr, Jones DJ Jr, Liddle GW & Dexter RN. Treatment of Cushing's disease. Correction of hypercortisolism by o,p'-DDD without induction of aldosterone deficiency. *New England Journal of Medicine* 1969 **281** 801–805. (doi:10.1056/NEJM196910092811501)
- 18 Luton JP, Cerdas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P, Bonnin A & Bricaire H. Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *New England Journal of Medicine* 1990 **322** 1195–1201. (doi:10.1056/NEJM199004263221705)
- 19 Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi G, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L & Berruti A. Adjuvant mitotane treatment for adrenocortical carcinoma. *New England Journal of Medicine* 2007 **356** 2372–2380. (doi:10.1056/NEJMoa063360)
- 20 Veytsman I, Nieman L & Fojo T. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for adrenocortical carcinoma. *Journal of Clinical Oncology* 2009 **27** 4619–4629. (doi:10.1200/JCO.2008.17.2775)
- 21 Luton JP, Mahoudeau JA, Bouchard P, Thieblot P, Hautecouverture M, Simon D, Laudat MH, Touitou Y & Bricaire H. Treatment of Cushing's disease by o,p'-DDD. Survey of 62 cases. *New England Journal of Medicine* 1979 **300** 459–464. (doi:10.1056/NEJM197903013000903)
- 22 Moolenaar AJ, van Slooten H, van Seters AP & Smeenk D. Blood levels of o,p'-DDD following administration in various vehicles after a single dose and during long-term treatment. *Cancer Chemotherapy and Pharmacology* 1981 **7** 51–54. (doi:10.1007/BF00258213)
- 23 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM & Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1526–1540. (doi:10.1210/jc.2008-0125)
- 24 Carrasco CA, Coste J, Guignat L, Groussin L, Dugue MA, Gaillard S, Bertagna X & Bertherat J. Midnight salivary cortisol determination for assessing the outcome of transsphenoidal surgery in Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4728–4734. (doi:10.1210/jc.2008-1171)
- 25 Yaneva M, Mosnier-Pudar H, Dugue MA, Grabar S, Fulla Y & Bertagna X. Midnight salivary cortisol for the initial diagnosis of Cushing's syndrome of various causes. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3345–3351. (doi:10.1210/jc.2003-031790)
- 26 Andersen A, Kasperlik-Zaluska AA & Warren DJ. Determination of mitotane (o,p'-DDD) and its metabolites o,p'-DDA and o,p'-DDE in plasma by high-performance liquid chromatography. *Therapeutic Drug Monitoring* 1999 **21** 355–359. (doi:10.1097/00007691-199906000-00020)
- 27 Laird NM & Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982 **38** 963–974. (doi:10.2307/2529876)
- 28 Terzolo M, Pia A, Berruti A, Osella G, Ali A, Carbone V, Testa E, Dogliotti L & Angeli A. Low-dose monitored mitotane treatment achieves the therapeutic range with manageable side effects in patients with adrenocortical cancer. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2234–2238. (doi:10.1210/jc.85.6.2234)
- 29 Baudin E, Pellegriti G, Bonnay M, Penfornis A, Laplanche A, Vassal G & Schlumberger M. Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'-DDD) levels on the treatment of patients with adrenocortical carcinoma. *Cancer* 2001 **92** 1385–1392. (doi:10.1002/1097-0142(20010915)92:6<1385::AID-CNCR1461>3.0.CO;2-2)

- 30 Faggiano A, Leboulleux S, Young J, Schlumberger M & Baudin E. Rapidly progressing high o,p'-DDD doses shorten the time required to reach the therapeutic threshold with an acceptable tolerance: preliminary results. *Clinical Endocrinology* 2006 **64** 110–113. (doi:10.1111/j.1365-2265.2005.02403.x)
- 31 von Slooten H, van Seters AP, Smeenk D & Moolenaar AJ. O,p'-DDD (mitotane) levels in plasma and tissues during chemotherapy and at autopsy. *Cancer Chemotherapy and Pharmacology* 1982 **9** 85–88. (doi:10.1007/BF00265384)
- 32 van Slooten H, Moolenaar AJ, van Seters AP & Smeenk D. The treatment of adrenocortical carcinoma with o,p'-DDD: prognostic implications of serum level monitoring. *European Journal of Cancer and Clinical Oncology* 1984 **20** 47–53. (doi:10.1016/0277-5379(84)90033-6)
- 33 Haak HR, Hermans J, van de Velde CJ, Lentjes EG, Goslings BM, Fleuren GJ & Krans HM. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. *British Journal of Cancer* 1994 **69** 947–951. (doi:10.1038/bjc.1994.183)
- 34 Benecke R, Keller E, Vetter B & de Zeeuw RA. Plasma level monitoring of mitotane (o,p'-DDD) and its metabolite (o,p'-DDE) during long-term treatment of Cushing's disease with low doses. *European Journal of Clinical Pharmacology* 1991 **41** 259–261. (doi:10.1007/BF00315440)
- 35 Schteingart DE, Tsao HS, Taylor CI, McKenzie A, Victoria R & Therrien BA. Sustained remission of Cushing's disease with mitotane and pituitary irradiation. *Annals of Internal Medicine* 1980 **92** 613–619.
- 36 Knappe G, Gerl H, Ventz M & Rohde W. The long-term therapy of hypothalamic-hypophyseal Cushing's syndrome with mitotane (o,p'-DDD). *Deutsche Medizinische Wochenschrift* 1997 **122** 882–886. (doi:10.1055/s-2008-1047704)
- 37 Patil CG, Veeravagu A, Prevedello DM, Katznelson L, Vance ML & Laws ER Jr. Outcomes after repeat transsphenoidal surgery for recurrent Cushing's disease. *Neurosurgery* 2008 **63** 266–270 (discussion 270–271). (doi:10.1227/01.NEU.0000313117.35824.9F)
- 38 Wagenmakers MA, Netea-Maier RT, van Lindert EJ, Timmers HJ, Grotenhuis JA & Hermus AR. Repeated transsphenoidal pituitary surgery (TS) via the endoscopic technique: a good therapeutic option for recurrent or persistent Cushing's disease (CD). *Clinical Endocrinology* 2009 **70** 274–280. (doi:10.1111/j.1365-2265.2008.03334.x)
- 39 Valassi E, Crespo I, Gich I, Rodriguez J & Webb SM. A reappraisal of the medical therapy with steroidogenesis inhibitors in Cushing's syndrome. *Clinical Endocrinology* 2012. In press. (doi:10.1111/j.1365-2265.2012.04424.x)
- 40 Vilar L, Naves LA, Azevedo MF, Arruda MJ, Arahata CM, Moura ESL, Agra R, Pontes L, Montenegro L, Albuquerque JL & Canadas V. Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease. *Pituitary* 2010 **13** 123–129. (doi:10.1007/s11102-009-0209-8)
- 41 Kamenicky P, Droumaguet C, Salenave S, Blanchard A, Jublanc C, Gautier JF, Brailly-Tabard S, Leboulleux S, Schlumberger M, Baudin E, Chanson P & Young J. Mitotane, metyrapone, and ketoconazole combination therapy as an alternative to rescue adrenalectomy for severe ACTH-dependent Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 2796–2804. (doi:10.1210/jc.2011-0536)
- 42 Daffara F, De Francia S, Reimondo G, Zaggia B, Aroasio E, Porpiglia F, Volante M, Termine A, Di Carlo F, Dogliotti L, Angeli A, Berruti A & Terzolo M. Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. *Endocrine-Related Cancer* 2008 **15** 1043–1053. (doi:10.1677/ERC-08-0103)
- 43 Donadille B, Groussin L, Waintrop C, Abbas H, Tenenbaum E, Dugue MA, Coste J, Bertagna X & Bertherat J. Management of Cushing's syndrome due to ectopic adrenocorticotropin secretion with 1,ortho-1, para'-dichloro-diphenyl-dichloro-ethane: findings in 23 patients from a single center. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 537–544. (doi:10.1210/jc.2009-1317)
- 44 Maher VM, Trainer PJ, Scoppola A, Anderson JV, Thompson GR & Besser GM. Possible mechanism and treatment of o,p'-DDD-induced hypercholesterolaemia. *Quarterly Journal of Medicine* 1992 **84** 671–679.

Received 30 April 2012

Revised version received 3 July 2012

Accepted 19 July 2012