THERAPY OF ENDOCRINE DISEASE

Steroidogenesis enzyme inhibitors in Cushing's syndrome

Eleni Daniel¹ and John D C Newell-Price²¹Department of Human Metabolism and ²Academic Unit of Endocrinology, Department of Endocrinology, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK

Correspondence
should be addressed
to J D C Newell-Price
Email
j.newellprice@sheffield.ac.uk

Abstract

Steroidogenesis enzyme inhibitors are the mainstay of medical therapy in Cushing's syndrome (CS). Ketoconazole (KTZ) and metyrapone are the most commonly used agents. Although there is considerable experience of their use in individual specialist centres, these drugs have not been rigorously tested in prospective clinical trials. Clinicians face uncertainties and concerns with respect to the safety profile of these agents, and best means to monitor effect. We review steroidogenesis inhibitors in the management of CS, including older agents (KTZ, metyrapone, etomidate and mitotane) and those currently under development (LCI699, non-racemic KTZ), and offer a practical approach for their use in clinical practice.

*European Journal of
Endocrinology*
(2015) **172**, R263–R280

Introduction

Cushing's syndrome (CS) has excess mortality and morbidity due to the long-term sequelae of cortisol excess (1). The primary aim of treatment is to reverse hypercortisolaemia and its devastating effects. Currently, surgery offers the only curative option by removing the source of adrenocorticotrophin (ACTH) or cortisol excess (2). Cure is not, however, always possible; in Cushing's disease (CD) remission rates following pituitary surgery are 40–90% even in specialist centres (2). Surgery may be deemed to be an unacceptable risk for some patients with adrenal or extra-adrenal CD and it may not be an option for others due to the lack of a definite surgical target on imaging.

This means that significant numbers of patients will need medical therapy to improve clinical and biochemical consequences of hypercortisolism (3).

Steroidogenesis enzyme inhibitors remain the mainstay for the medical treatment of hypercortisolaemia of CS, with extensive clinical experience, albeit not always published, of these agents (4, 5, 6, 7, 8). In the modern era, ketoconazole (KTZ) and metyrapone are most commonly used, with well-characterised pharmacological properties, acting on the adrenal cortex to inhibit various steps of the steroid biosynthetic pathway and causing reversible reduction in cortisol synthesis. The action of mitotane is

Invited Author's profile:

John D C Newell-Price is Professor of Endocrinology at the University of Sheffield. John has been at the University of Sheffield since 2000 and is the Clinical Lead for the endocrine service at the Sheffield Teaching Hospitals NHS Foundation Trust. He chairs The Royal College of Physicians, UK Joint Specialist Committee for Diabetes and Endocrinology, and the Medical Committee of the Pituitary Foundation, where he is also a trustee. His research interests include glucocorticoids, pituitary and neuroendocrine tumours. He currently serves on task forces for clinical guidelines for the American Endocrine Society and the European Society of Endocrinology.

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R264
--------	------------------------------------	--	-------	------

less well defined, and it is used less commonly in benign disease; it may have an irreversible action with sufficient dose and prolonged use. The use of parenteral etomidate is limited to specialist units and small patient numbers (9).

Recently, use of the steroidogenesis enzyme inhibitors has been re-evaluated. Safety concerns regarding the hepatotoxicity of KTZ have led to limited availability in many countries (5). At the same time, there is a potential for new inhibitors to enter clinical practice over the next few years (10). Outside this class of agents, there are limited new alternatives. Pasireotide, a somatostatin analogue, and mifepristone, a glucocorticoid receptor antagonist, have recently been approved for use in CD and CS causing diabetes, respectively, following formal prospective interventional studies (11, 12, 13). The steroidogenesis inhibitors have never been subjected to the rigors of a prospective clinical trial, and efficacy and side effects may be over and under estimated, respectively, but, nevertheless, they remain as important agents for the management of hypercortisolaemia in all forms of CS. Here, we review steroidogenesis inhibitors used in current practice and give guidance for their use in the management of CS and also highlight those under development.

Currently available steroidogenesis inhibitors

Ketoconazole

KTZ is an imidazole derivative, initially developed as an orally active antifungal agent (14, 15). It was introduced in clinical practice for the treatment of systemic fungal infections in the early 1980s (16, 17). In fungi, it inhibits the synthesis of ergosterol, a cell membrane sterol. Shortly after its introduction as an antifungal agent, reports linked long-term KTZ administration with the development of gynaecomastia in men (18) and low serum testosterone levels (19, 20). Subsequently, Pont *et al.* (21) reported glucocorticoid suppression with KTZ both *in vitro* and *in vivo*, confirming its inhibitory effect on adrenal steroidogenesis.

In the adrenal cortex, KTZ blocks multiple steps of steroid biosynthesis through the inhibition of cytochrome p450 enzymes 17 α -hydroxylase, 20,22-desmolase (22), 11 β -hydroxylase (22, 23, 24) 17,20-desmolase (24, 25) and 18-hydroxylase (26) (Fig. 1; Table 1). Similarly, it blocks various steps of steroidogenesis in the gonads (27, 28, 29), leading to the potential side effect of hypogonadism in men, if used in the long term.

Following the discovery of the effect on adrenal steroidogenesis, KTZ was used to treat conditions of

cortisol excess, and several small studies demonstrated clinical and biochemical response in patients with CS (Table 2). Early reports were very encouraging. In one of the earlier studies, all five patients with recurrent CD following pituitary surgery had rapid biochemical and clinical response within 4 weeks of treatment (30). Larger, single-centre retrospective studies confirmed the efficacy: Sonino *et al.* (31) reported the use of KTZ in 34 patients with CS (28 had CD), which resulted in the normalisation of urinary free cortisol (UFC) in 88% (30 out of 32 patients) with significant improvements in blood pressure, hypokalaemia, glycaemic control and hirsutism. Somewhat less favourable data from France in 38 patients with CS demonstrated control in 45% of patients, but with 24% not improving (32). The largest retrospective study on the use of KTZ in CS involved 200 patients from 14 centres in France. Castinetti *et al.* reported a response rate of 75.3% with 49% of patients achieving normalisation of UFC. The effectiveness in clinical practice is likely to be an underestimate because one-third of the patients with incomplete control on long-term therapy were receiving less than the maximal daily dose. Other reasons for suboptimal dosing could be poor tolerability, which was the reason for withdrawal of treatment in 20.5% of patients. These data indicate that whilst the majority of patients had clinical and biochemical improvement, aggressive up-titration of dose is needed to maximise the efficacy of the drug (5). To date in the English literature 456 patients with CS have been treated with KTZ monotherapy, with or without surgery or radiotherapy: 60% of patients achieved normalisation of UFC with or without complete remission of the clinical features (23, 33, 34, 35).

The usual dosage of KTZ for the management of CS is 400–1600 mg daily, significantly higher than the recommended dose for its antifungal activity (200–400 mg/daily) (Table 3). For optimal absorption, the presence of an acidic gastric environment is necessary (16). Achlorhydria, administration on an empty stomach or concurrent use of antacids and proton pump inhibitors may lead to reduced absorbance and treatment failure (16, 27, 36). In these cases, a dose increase may be needed to achieve therapeutic plasma concentrations. Ideally antacids should be stopped but if necessary to continue, it has been shown that absorbance is enhanced when administered together with an acidic beverage (cola drink or equivalent) or an acidified (hydrochlorified) solution (36, 37). Side effects of KTZ are common and include gastrointestinal symptoms, hepatotoxicity, allergic reactions, male hypogonadism and gynaecomastia (32).

Figure 1

Steroidogenesis in the adrenal cortex denoting the specific pathways inhibited by ketoconazole (KTZ), metyrapone (MTR), mitotane, etomidate and newer steroidogenesis inhibitors

(17α-OH, 17α-hydroxylase; 3βHSD, 3β-hydroxysteroid dehydrogenase; 21-OH, 21-hydroxylase; 11β-OH, 11β-hydroxylase; 18-OH, 18-hydroxylase; *aldosterone synthase).

In 2013/2014, the use of KTZ as an antifungal agent has been restricted mainly due to concerns of life-threatening hepatotoxicity. In Europe, following a review of all available safety data, the European Medicines Agency recommended suspension of the marketing authorisations for oral KTZ for the treatment of fungal infections as the risk of liver injury outweighs its therapeutic benefits (38). Similarly, in the USA, the Food and Drug Administration has restricted its use for only severe fungal infections in the absence of other alternatives and approved label changes regarding indications, drug interactions and the potential for adrenal suppression (39).

Reports on hepatotoxicity appeared early on following introduction of KTZ for the treatment of fungal infections (40, 41) and hypercortisolaemia (33). In the majority of cases, KTZ caused an asymptomatic increase in liver transaminases; however, fatal hepatitis has been reported in patients receiving antifungal doses (200 mg daily) after both short- and long-term treatment courses (42).

Commonly, the increase in liver transaminases occurs within 4 weeks of initiation of treatment or a dose change (5) and it is recommended that liver function tests should be checked regularly at the start and throughout the duration of therapy. Cessation is recommended when transaminases increase by more than three times the upper limit of normal (40). The substitution of KTZ by newer and safer antifungal agents for the management of fungal

Table 1 Steroidogenesis enzymes and their genes.

Gene	Protein
<i>CYP11A1</i>	20,22-desmolase or cholesterol side-chain cleavage enzyme
<i>HSD3B2</i>	3β-hydroxysteroid dehydrogenase
<i>CYP17A1</i>	17α-hydroxylase/17,20-desmolase
<i>CYP21A2</i>	21-hydroxylase
<i>CYP11B1</i>	11β-hydroxylase
<i>CYP11B2</i>	Aldosterone synthase (11β-hydroxylase/18-hydroxylase)

Table 2 Studies investigating the use of ketoconazole in Cushing's syndrome where ketoconazole is the only medical therapy. The studies are retrospective and include at least five patients.

Reference	Number of patients	CD	Daily dose (mg)	Age	Duration of treatment	Biochemical test	Normalisation (%)	Improvement in clinical features	Notes	Side effects (number of patients)
(34)	5		800	20–44	More than 4m	UFC, early morning cortisol		All improved, including menstrual problems		Liver toxicity (1)
(30)	5	5	600–800	18–48	2–6m	UFC, early morning cortisol	100	All improved (BP, glycaemic control, menstrual problems, hirsutism in all women)	All patients had pituitary surgery before and four had pituitary RT shortly after starting KTZ	Gynaecomastia (1)
(35)	8	7	600–800	16–48	3–13m	UFC, plasma cortisol	75	Yes	Pituitary surgery	0
(155)	6		600	30–55	4–6w				No previous surgery	
(156)	6	6	800	26–69	1w	UFC	83			4, reversible liver toxicity (3)
(157)	9	8	600		1w to 1y	UFC, serum cortisol	33	Slight improvement in 1	Some had pituitary surgery	
(24)	8		800		2w		100	Yes in all (BP, glycaemic control and hypokalaemia)		Liver toxicity (2)
(158)	8	4	400–1200		3–6m	UFC	50	Yes, BP (20/21), glycaemic control, hirsutism, myalgia, psychiatric problems, potassium replacement stopped (6/8), hirsutism		0
(31)	34	28	400–1200	14–67	More than 6m (n=12), up to 6m (n=18)	UFC, early morning cortisol	88	Yes BP, glycaemic control, hypokalaemia and myalgia improved in the majority	Nine had chemotherapy as well	5, liver toxicity (4)
(159)	15		400–1200	44–84	3d to 35m	UFC	47	Yes BP, glycaemic control, hypokalaemia and myalgia improved in the majority		
(56)	6	6			6w to 29m		83		Macroadenomas, all patients had previous surgery(ies)	Liver toxicity (2)
(160)	5	3		> 75				Yes		

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R267
--------	------------------------------------	--	-------	------

Table 2 Continued.

Reference	Number of patients	CD	Daily dose (mg)	Age	Duration of treatment	Biochemical test	Normalisation (%)	Improvement in clinical features	Notes	Side effects (number of patients)
(32)	38	38	200–1200	18–73	6–72m, mean 23m	UFC	45	Yes (BP and glycaemic control)	All patients had pituitary surgery in the past	Intolerant of therapy (6), liver toxicity (5), nausea (2)
(161)	17		200–1000	7–63	1–30.7m	UFC	53	Yes (BP and glycaemic control)	CS not previously treated	Liver toxicity (3)
(5)	200	200	200–1200	8–87		UFC	49	Yes in 88/172 (49/116 BP, 31/55 glycaemic control and 15/39 hypokalaemia)	In 128, previous surgery or RT	85 events, intolerant of treatment (41), reversible liver toxicity (3)

CD, Cushing's disease; CS, Cushing's syndrome; UFC, urinary free cortisol; BP, blood pressure; RT, radiotherapy; y, year; m, months; w, weeks; d, days; KTZ, ketoconazole.

infections is justified; however, when it comes to treating patients with hypercortisolaemia, the risk:benefit ratio differs. Given the paucity of alternative options and the proven efficacy in a number of patients, use of KTZ can be justified and has been approved for this purpose by the European Medicines Authority in late 2014. Close monitoring in centres with appropriate experience is vital, but the consequence of the change in the prescribing guidance is that KTZ has become less easily available (43).

Metyrapone

Metyrapone (2-methyl-1,2-bis-(3-pyridyl)-1-propanone) was developed as a potential steroidogenesis inhibitor and was first described by Liddle *et al.* in 1958 (44). It has a rapid onset of action and a short half-life of about 2 h (6). It is administered orally and absorbed quickly, achieving peak plasma concentrations within 1 h of ingestion. Although it is a potent and relatively selective inhibitor of 11β-hydroxylase, it also inhibits 18-hydroxylase (Fig. 1), and recent *in vitro* data indicate that overall it has greater inhibitory action on aldosterone synthase, a feature not previously recognised (45). Oral administration of metyrapone results in a significant and reversible reduction in cortisol and aldosterone production (46). The loss of negative feedback due to low circulating cortisol levels may then lead to an increase in ACTH levels which drives the accumulation of cortisol and aldosterone precursors (predominantly 11-deoxycorticosterone, 11-deoxycortisol) (47) and androgens in the blood and urine (4, 44, 46, 47). The accumulation of precursors explains some of the side effects associated with this treatment.

In CD reduction in cortisol level due to metyrapone therapy causes a compensatory increase in ACTH, which may then drive further steroidogenesis and overcome the blockade. For this reason metyrapone was initially considered to be ineffective for CD. Therefore, it was primarily used as a test for pituitary–adrenal reserve and the differential diagnosis of ACTH-dependent CS (48, 49, 50). Later studies in 18 patients with CS treated with a combination of metyrapone and aminoglutethimide, an antiepileptic and steroidogenesis enzyme inhibitor, indicated that metyrapone may be effective in improving clinical and biochemical features of CS (51). Subsequently, Jeffcoate *et al.* (52) reported treatment of 13 patients with CD with a dose of 0.5–4 g of metyrapone daily; the patients showed good clinical and biochemical improvement on metyrapone despite a rise in plasma ACTH levels. Metyrapone was thus shown to control

CD, Cushing's disease; CS, Cushing's syndrome; UFC, urinary free cortisol; BP, blood pressure; RT, radiotherapy; y, year; m, months; w, weeks; d, days; KTZ, ketoconazole.

infections is justified; however, when it comes to treating patients with hypercortisolaemia, the risk:benefit ratio differs. Given the paucity of alternative options and the proven efficacy in a number of patients, use of KTZ can be justified and has been approved for this purpose by the European Medicines Authority in late 2014. Close monitoring in centres with appropriate experience is vital, but the consequence of the change in the prescribing guidance is that KTZ has become less easily available (43).

Metyrapone

Metyrapone (2-methyl-1,2-bis-(3-pyridyl)-l-propanone) was developed as a potential steroidogenesis inhibitor and was first described by Liddle *et al.* in 1958 (44). It has a rapid onset of action and a short half-life of about 2 h (6). It is administered orally and absorbed quickly, achieving peak plasma concentrations within 1 h of ingestion. Although it is a potent and relatively selective inhibitor of 11 β -hydroxylase, it also inhibits 18-hydroxylase (Fig. 1), and recent *in vitro* data indicate that overall it has greater inhibitory action on aldosterone synthase, a feature not previously recognised (45). Oral administration of metyrapone results in a significant and reversible reduction in cortisol and aldosterone production (46). The loss of negative feedback due to low circulating cortisol levels may then lead to an increase in ACTH levels which drives the accumulation of cortisol and aldosterone precursors (predominantly 11-deoxycorticosterone, 11-deoxycortisol) (47) and androgens in the blood and urine (4, 44, 46, 47). The accumulation of precursors explains some of the side effects associated with this treatment.

In CD reduction in cortisol level due to metyrapone therapy causes a compensatory increase in ACTH, which may then drive further steroidogenesis and overcome the blockade. For this reason metyrapone was initially considered to be ineffective for CD. Therefore, it was primarily used as a test for pituitary–adrenal reserve and the differential diagnosis of ACTH-dependent CS (48, 49, 50). Later studies in 18 patients with CS treated with a combination of metyrapone and aminoglutethimide, an antiepileptic and steroidogenesis enzyme inhibitor, indicated that metyrapone may be effective in improving clinical and biochemical features of CS (51). Subsequently, Jeffcoate *et al.* (52) reported treatment of 13 patients with CD with a dose of 0.5–4 g of metyrapone daily; the patients showed good clinical and biochemical improvement on metyrapone despite a rise in plasma ACTH levels. Metyrapone was thus shown to control

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R268
---------------	------------------------------------	--	-------	-------------

Table 3 Proposed dosage and monitoring regimens for the steroidogenesis inhibitors in the management of hypercortisolaemia.

	Dosage	Biochemical monitoring
Ketoconazole	Starting dose 400 mg can increase up to 1600 mg daily in two or three divided doses	1. UFC or cortisol day-curve at day 3, 8, 14, 21 and 35. Adjust dose if needed and recheck in 1 week 2. Liver function tests weekly for the first month of treatment and then monthly
Metyrapone	Starting dose for CD or benign adrenal disease is 750–1000 mg in three divided doses and for EAS or ACC is 1500 mg in three or four divided doses	1. UFC or cortisol day-curve at day 3, 8, 14, 21 and 35 2. Adjust dose according to biochemical response by 250–500 mg per dose and recheck biochemistry in 1 week
Etomidate	2.5 mg/h or 0.03–0.05 mg/kg per h and i.v. hydrocortisone infusion (1–3 mg/h) or i.m. dexamethasone (5, 86)	1. Check serum cortisol daily and after every dose change 2. Nurse in a High Dependency Unit and monitor for sedation 3. Adjust etomidate dose according to cortisol-lowering effect and level of sedation
Mitotane	High-dose regimen: start with 1.5 g daily and increase dose by 1.5 g every 24 h until a dose of 6 g Low-dose regimen: start with 1 g daily and increase by 0.5 g every 72 h to a dose of 3 g daily Maximum dose is 12 g but high doses may not be tolerated	1. Check mitotane plasma levels at 2, 4, 6, 8, 10 and 12 weeks initially, then monthly until levels are stable. Thereafter every 2–3 months. For anti-tumour effect target levels are 14–20 mg/l. For Cushing's disease target levels 8.5–20 mg/l 2. Make adjustments of the dose by 1.5 g according to tolerance and plasma levels. If levels > 20 mg/l a more significant decrease in the dose by 50–80% may be needed. If significant neurological side effects develop mitotane should be stopped 3. Use double-dose steroid replacement and avoid dexamethasone as the replacement glucocorticoid 4. Monitor TFTs 5. Monitor hypogonadism clinically

hypercortisolism in anticipation of more permanent treatment such as pituitary radiotherapy in adults (53) and children (54, 55).

Several small retrospective series and case reports further explored the efficacy of metyrapone alone or in combination with KTZ (56) (Table 4). In the largest study to date, Verhelst reported the response of 91 patients with CS treated by metyrapone monotherapy. Newly diagnosed, previously untreated CD patients who received metyrapone for <16 weeks achieved biochemical normalisation in 75% on a median dosage of 2250 mg. Long-term response in patients with CS (ectopic ACTH syndrome (EAS) $n=18$, CD undergoing pituitary radiotherapy $n=24$, adrenal adenoma $n=10$, adrenocortical carcinoma $n=6$) was 70–83% and the medication was well-tolerated (6). Clinical improvement in myopathy, psychiatric problems, blood pressure and biochemical correction of hypokalaemia was observed in over 70% of patients (6). The data from a recent large multicentre study in the UK confirm similar efficacy (7). A search of the published English literature reveals just over 200 cases of patients with CS treated with metyrapone monotherapy with normalisation of hypercortisolism, as defined by the authors, in 75% of cases.

Side effects are common but usually mild and well tolerated. The most frequent side effects are gastrointestinal (which improve if the medication is administered with

a glass of milk or a small snack) and hypoadrenalism. A significant overlap may exist between the two conditions. Accumulation of androgens may cause hirsutism in women and worsening of acne. Hirsutism was reported in five out of seven women treated for longer than 6 months in early studies; however, only one patient reported severe symptoms (6, 52). Furthermore accumulation of 11-deoxycorticosterone, a steroid precursor with weak mineralocorticoid activity, may cause oedema and hypertension. Hypokalaemia is theoretically a potential side effect; however, it was not found to be a significant issue in a large retrospective study (6).

Mitotane (OP'-DDD)

Mitotane is an adrenolytic agent with steroidogenesis inhibitor activity at low doses (4, 57, 58). It is a synthetic derivative of the pesticide dichlorodiphenyltrichloroethane, which has toxic effects in the adrenal cortex of dogs (9, 59, 60). It is licenced for the symptomatic treatment of advanced or inoperable adrenocortical carcinoma (functional or not) but has also been used off-label for the management of hypercortisolaemia in CS (8, 61, 62, 63).

The exact mechanism of action is not clear. It is thought to inhibit multiple steroidogenesis enzymes, mainly the 20,22-desmolase, and possibly other steps

Table 4 Studies investigating the use of metyrapone in Cushing's syndrome where metyrapone is the only medical therapy. The studies are retrospective and include at least five patients.

Reference	Number of patients	CD	Dose of metyrapone (g)	Age	Duration of treatment	Biochemical test	Normalisation (%)	Improvement in clinical features	Notes	Side effects (number of patients)
(52)	13	13	0.500–4	16–60	2–66m	Plasma cortisol day-curve		Yes (facial features, skin, psychiatric symptoms, BP)	Pituitary RT (8 out of 13 patients)	Hirsutism or persistence of acne (5), intolerant to therapy (1)
(138)	18	The majority	0.500–6	15–60				Yes – psychiatric symptoms	Pituitary RT (15 out of 18 patients with CD) Mean UFC values significantly improved during treatment	
(53)	5	5		28–58	12–24m	UFC				
(6)	91	57	0.750–66		Short term up to 16w, long-term up to 140m	Serum cortisol day-curve	77	Yes, including hirsutism	Short-term group were not previously treated, long-term CD group had RT (24)	Hypoadrenalism, hirsutism
(162)	13		0.750–3	58.4 ± 19.0	6–238m	Serum cortisol	100	Yes, all (BP, glycaemic control, hypokalaemia)	All patients had EAS, 8 received RT and chemotherapy	
(161)	23		0.750–4	7–70	1–30.7m	UFC	57	Yes (HTN, glycaemic control)	85% had ACTH-dependent CS	Hirsutism (6), HTN, oedema (9)

CD, Cushing's disease; CS, Cushing's syndrome; EAS, ectopic ACTH syndrome; m, months; w, weeks; UFC, urinary free cortisol; BP, blood pressure; RT, radiotherapy.

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R270
--------	------------------------------------	--	-------	------

such as 11 β -hydroxylase, 18-hydroxylase and 5 α -reductase (64, 65, 66) (Fig. 1). It causes a reduction in cortisol and adrenal androgens levels, but the aldosterone production is not significantly affected with short-term treatment (67, 68). The metabolites of mitotane have a direct toxic effect on the mitochondria of the adrenal cells causing cellular necrosis (69, 70, 71, 72). Macroscopically, this mainly affects the zona reticularis and zona fascicularis, leaving the zona glomerulosa relatively spared (73, 74). Daily doses more than 4 g cause a non-reversible chemical adrenalectomy (75). Mitotane is lipophilic and accumulates in the adipose tissue (76), therefore its onset of action may be delayed for several weeks, during which time the control of hypercortisolism must be achieved with an alternative drug. Due to slow release from adipose tissue it has a long half-life (18–150 days) and may continue to exhibit the anti-adrenal effects for several months following cessation of therapy (76).

Mitotane has been used for the management of CS and there are only a few retrospective studies for this indication. It is essential to monitor plasma levels of mitotane. Schteingart *et al.* (67) described the remission of 29 out of 36 (81%) patients treated with mitotane and pituitary irradiation for CD: mitotane was started at a dose of 4 g daily and reduced slowly to a dose eventually as low as 500 mg twice weekly, with the monitoring of plasma cortisol and UFC for up to 7 years. Based on the patients' cortisol secretion rate, the authors identified that some patients were early responders (<4 months) and some others were late responders (>5 months). Interestingly, the two groups were not different in terms of the severity of hypercortisolism, total daily dose or plasma levels of mitotane (67). Recently, Baudry *et al.* have reported the use of mitotane in 76 patients with CD, the majority of which received mitotane as primary therapy and were not at the time of the study treated with pituitary radiotherapy. Overall, 72% of patients went into remission with mitotane after a median of 7 months. Plasma mitotane concentrations were inversely correlated with UFC and there was no abnormal concomitant UFC when the plasma levels were higher than 8.5 mg/l (8). Upon the withdrawal of mitotane, 71% of patients who did not receive alternative therapy experienced a relapse after 5–68 months (median 13.2 months). A high ACTH level at discontinuation was associated with a lower risk of relapse (8).

Gastrointestinal symptoms, including anorexia, nausea, vomiting and diarrhoea, are the most frequently reported side effects. Neurotoxicity (depression, dizziness/lethargy, confusion, headache, paraesthesia, ataxia, dysarthria) may be severe and necessitate discontinuation

of treatment, but tend to occur only when plasma levels >20 mg/l. Other common side effects are hypogonadism (due to inhibition of 5 α -reductase), gynaecomastia, hepatotoxicity and hypercholesterolaemia (that responds to statin therapy or the co-administration of KTZ) (8, 67, 77, 78). Frequently, the development of side effects can be managed by a small dose reduction (78).

Etomidate

Etomidate is an imidazole derivative that was initially developed in 1965 as a potential chemotherapeutic agent (79). Early studies in animal models, however, demonstrated that it induced rapid-onset reversible sedation and hypnosis in rats (79). It was therefore introduced to clinical practice as an anaesthetic agent in 1972. As a hypnotic agent, it has a unique safety profile with a rapid-onset and short duration of action as well as a very high therapeutic ratio (lethal dose is 12 times the hypnotic dose) compared with other hypnotics such as barbiturates (79).

Shortly after its introduction to intensive care units, reports linked the use of continuous infusions of etomidate with excess mortality and symptomatic adrenal insufficiency (80, 81, 82, 83). Subsequently, *in vitro* (84, 85) and *in vivo* studies in healthy subjects demonstrated a dose-dependent inhibition of the baseline and ACTH-stimulated cortisol secretion following etomidate infusions (82, 85) and single bolus injections (86). The cortisol-lowering effect of etomidate resulted in it being used less often for general anaesthesia induction, but this effect was also quickly recognised as being useful for the treatment of hypercortisolism. Due to its favourable cardiovascular and safety profile, etomidate is still used by many anaesthetists as an induction agent in emergency intubation and cardiac surgery (87, 88, 89, 90).

Etomidate is a potent inhibitor of 11 β -hydroxylation and to a lesser degree 20,22-desmolase (26, 91, 92) (Fig. 1). It is administered intravenously and has a very rapid onset of action. It causes reversible inhibition of 11 β -hydroxylase with blunting of the cortisol response to ACTH and increase in cortisol precursors (11-deoxycortisol) within half an hour following a single sub-anaesthetic dose (0.04 mg/kg) (93). As a hypnotic agent it has a short duration of action, but adrenal suppression may persist for several hours following a single dose or for several days following a continuous infusion (94). Prolonged administration causes persistent but reversible adrenal suppression and possibly a more significant proximal inhibition of the steroidogenesis pathway (95, 96). Higher doses may also

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R271
--------	------------------------------------	--	-------	------

cause a more prominent inhibition of the proximal steps of the steroid biosynthetic pathway than lower doses (97).

There are very limited reports on the use of etomidate in the treatment of hypercortisolaemia. Schulte *et al.* demonstrated that etomidate at a dose of 0.3 mg/kg per h for 24 h improved cortisol levels in six patients with CS and caused a dose-dependent decrease in cortisol levels in 15 eucortisolaemic subjects. This effect was observed even at low doses of etomidate, indicating that doses as low as 0.1 mg/kg per h may cause a clinically significant inhibition of adrenal steroidogenesis (92). Allolio *et al.* (98) reported that short-term (32 h) use of etomidate in non-hypnotic doses (2.5 mg/h) in six patients with CS improved cortisol and did not cause any sedative side effects compared with a higher dose of 0.3 mg/kg per h. Prolonged treatment with a block-and-replace regimen (etomidate 1.2–2.5 mg/h and replacement hydrocortisone infusion) was reported by Drake *et al.* (95) in a patient with EAS due to a pancreatic islet cell tumour who was unable to take enteral therapy whilst in the intensive care unit: there were no sedative or metabolic side effects in this patient during the 8 weeks of treatment. Recently, Soh *et al.* (99) have reported a protocol using etomidate infusions outside the critical care setting, which involves low non-sedative doses (3 mg/h) and administration through peripheral venous access.

Due to its central actions, etomidate may cause sedation, myoclonus and hypotension and needs administration in a highly monitored environment such as a Critical Care Unit setting. However, the antisteroidogenic effect also occurs at doses lower than those needed for the sedative or hypnotic effects (92, 100). For this reason, a lower dosage of etomidate may be used to avoid sedation when it is not otherwise needed (99), although there are anecdotal reports of its ambulatory use delivered by a portable syringe driver in the occasional patient with adrenal cortical cancer and severe CS.

There are two available formulations of etomidate: a lipid emulsion preparation and an aqueous solution of 35% propylene glycol. Toxicity due to propylene glycol may occur in high doses and the WHO suggests that the maximum oral safe intake is 25 mg/kg daily (101). Although propylene glycol is administered intravenously in the case of etomidate administration, propylene toxicity is unlikely to occur in the low doses needed for cortisol suppression and it appears to be safe even at higher doses in situations where sedation for ventilation was desired as well (0.06–0.03 mg/kg per h) (102). Prolonged administration (5.5 months) of the propylene glycol preparation of etomidate was reported in a 39-year-old patient with the EAS who was unable to have oral medications; in order to reduce the total

dose needed, etomidate was given as a dose-titration regimen and the dose was adjusted during an episode of acute renal failure with no significant adverse effects (96). Attention should be given to the total dose of propylene glycol and the presence of potential signs of toxicity (unexplained metabolic acidosis and a high anion gap, haemolysis, renal failure, liver dysfunction, CNS depression, cardiac arrhythmias) (103).

New agents under investigation

LCI699

LCI699 is the first orally active inhibitor of aldosterone synthase (CYP11B2 product), developed as a potential treatment for hypertension, cardiac failure and renal disease. In early clinical trials, it was observed that while at low doses it is a potent and selective inhibitor of aldosterone synthetase, at higher doses it also inhibits 11 β -hydroxylase (CYP11B1 product) (104, 105). Following treatment with LCI699, hypertensive patients, either due to primary hyperaldosteronism or essential hypertension, showed a reduction in blood pressure associated with a dose-dependent decrease in plasma and urine aldosterone (up to 80%) and an increase in 11-deoxycorticosterone (104, 105, 106). At doses of 0.5–1 mg, daily there was an increase in steroid precursors 11-deoxycorticosterone and 11-deoxycortisol and a blunting of the cortisol response to synthetic ACTH, indicating inhibition of 11 β -hydroxylase (104, 105).

Initial antihypertensive trials indicated only a modest inhibition of aldosterone and reduction in blood pressure at doses that were safe to avoid significant cortisol suppression. Bertagna *et al.* subsequently explored the use of this new agent in the management of hypercortisolism. Using substantially higher doses than those used for aldosterone synthase inhibition (4–100 mg daily vs up to 3 mg daily), LCI699 was administered in a dose escalation study in 12 patients with CD (the majority had a macroadenoma) and previous pituitary surgery. The primary endpoint was the normalisation of UFC on therapy and 92% of patients achieved this within 10 weeks of therapy (10). All patients responded by a reduction in UFC level of more than 50% (UFC was 4.6-fold ULN at baseline) (10). Mean systolic and diastolic blood pressure improved; however, patients developed weight gain (average 2.4 kg) and one patient developed significant oedema. There was a three- to fourfold increase in ACTH and a significant increase in 11-deoxycortisol and 11-deoxycorticosterone during treatment, the latter explaining the weight gain and oedema. Women had a

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R272
--------	------------------------------------	--	-------	------

significant but transient increase in testosterone at week 8. A clinical trial with longer-term therapy is on-going and an interim analysis of the first eight patients showed a response rate of 75% after 22 weeks (107).

LCI699 is absorbed rapidly with a peak blood concentration 1 h after oral administration and a half-life of 4–5 h (108, 109). The few available studies indicate that it is generally well tolerated. Side effects include hypernatraemia, gastrointestinal effects (nausea, diarrhoea), fatigue, headache, oedema and hypokalaemia (10, 107, 109). Patients with primary hyperaldosteronism treated with small doses of LCI699 showed improvement of hypokalaemia and were able to discontinue potassium supplements. At the high doses needed to treat hypercortisolaemia, hypokalaemia was a problem in 30% of patients and a small decrease in potassium levels was observed overall (10, 105).

LCI699 shows promising results in terms of controlling hypercortisolaemia and is the most potent inhibitor of 11 β -hydroxylase currently available. However, it causes an increase in ACTH that drives significant 11-deoxycorticosterone increase, and long-term studies are important to establish the side-effect profile in terms of its mineralocorticoid effects.

Non-racemic KTZ (2S,4R KTZ)

KTZ is a racemic mixture of two *cis*-enantiomers 2S,4R and 2R,4S. Stereoisomers may have different pharmacological properties and an evaluation of the activity of the two enantiomers against cytochrome p450 enzymes showed that 2S,4R KTZ has higher activity against steroidogenic enzymes such as 11 β -hydroxylase, 20,22-desmolase and 17 α ,20 lyase (110). These results indicate that it is possible that a non-racemic mixture of KTZ could be more efficacious in reducing cortisol levels and safer in terms of hepatotoxicity. The pure enantiomer 2S,4R was tested in individuals with type 2 diabetes and was shown to improve the measures of glycaemic control and lipids (111); however, the development of this drug for type 2 diabetes was terminated due to an unacceptable safety profile (112). The 2S,4R enantiomer is currently being tested in patients with CS in a Phase 3 trial (113).

How to use the steroidogenesis enzyme inhibitors

Choice of agent

Both metyrapone and KTZ have been used in adult men and women of all ages. The decision of which treatment is more

appropriate should be individually tailored taking into account the known side effects. For example, hypogonadism and gynaecomastia are well-documented side effects of long-term KTZ therapy; therefore, metyrapone is a rational first choice in young men. In women, androgenic side effects (hirsutism, peripheral oedema) due to metyrapone may eventually lead to discontinuation of therapy and patients should be appropriately counselled about this side effect, and KTZ may be a more rational choice for long-term treatment in women. Mitotane is teratogenic and is contraindicated in women desiring fertility (114).

There are no direct head-to-head comparison studies of KTZ and metyrapone. Prolonged administration of either metyrapone or KTZ appears safe and should be considered in cases where other options (surgery or radiotherapy) have been exhausted or where there is a strong patient preference for a non-surgical approach (115). The efficacy of medical therapy appears to be better in long-term treated groups and this may be associated with the fact that most patients by that stage have undergone other therapies that have reduced the disease burden.

Etomidate is the only parenteral active steroidogenesis inhibitor and should be used in cases where rapid control of hypercortisolaemia is vital and an oral agent is contraindicated (9, 102, 116, 117, 118). In addition, it can be very useful in situations where sedation is a desired effect, such as patients requiring intubation for respiratory support or patients with acute psychosis.

Pregnancy

Fertility is impaired in young women with CS, therefore a new diagnosis of this condition during pregnancy is extremely rare. Hypercortisolaemia can interfere with the secretion of gonadotrophins causing anovulation and amenorrhoea (119, 120), and hyperandrogonaemia may interfere with implantation. There are very few case reports of women who developed hypercortisolaemia and were diagnosed during pregnancy; biochemical diagnosis is challenging due to the physiological hormonal changes and lack of clearly defined pregnancy reference ranges (121, 122, 123).

Women on medical therapy for CS may also become pregnant or women may relapse during pregnancy (124, 125). Medical or surgical treatment has complications but so does uncontrolled hypercortisolism, and treatment should be individualised, taking into account the gestational age of the pregnancy. Surgery is the preferred option, when it is safe during pregnancy or following delivery, while medical therapy is used to control

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R273
--------	------------------------------------	--	-------	------

hypercortisolism until surgery can be performed. Untreated maternal hypercortisolaemia has significant implications on maternal and foetal health and there is evidence that treatment improves the rate of live births (122, 126). There are no approved medical treatments for use in pregnancy; however, there are several reports of the use of metyrapone or KTZ in this difficult clinical scenario.

The most commonly reported medication in pregnancy is metyrapone. There are concerns that metyrapone may affect the biosynthesis of steroids in the placenta; however, several case reports describe its use in the first (127, 128, 129), second (123, 127, 129, 130, 131, 132) and third trimesters (123, 126, 129, 130, 133, 134). Metyrapone is generally well tolerated and doses up to 3 g have been used to control hypercortisolaemia (123, 130). It is not thought to be teratogenic and there are reports of administration in the 1st trimester during foetal organogenesis with no reports of congenital anomalies (128, 129). A block-and-replace approach is usually used although dose titration has also been used (130). Metyrapone and its active metabolite rac-metyrapol are excreted in maternal milk with commonly used doses (250 mg qds) but at levels that are extremely unlikely to be a significant risk for the neonate (134). Side effects in pregnancy include hypertension and pre-eclampsia and pre-existing hypertension appears to be a risk for pre-eclampsia for women taking metyrapone (132, 135).

There are fewer case reports on the use of KTZ in pregnancy mainly because of concerns of hepatotoxicity and the potential for the inhibition of androgens due to the aromatase activity. Lack of androgens in a male foetus may affect sexual differentiation resulting in ambiguous genitalia and cryptorchidism. Amado *et al.* (136) reported the use of KTZ in the 3rd trimester in a 36-year-old woman with CS due to an adrenocortical adenoma; doses up to 600 mg daily were given between 32 and 37-week gestation of a female foetus, resulting in a significant reduction in UFC and no maternal or neonatal complications. There have been reports of uncomplicated pregnancies and live births of healthy male babies in women who were treated with KTZ at conception and throughout the first trimester (124, 125).

Mitotane is contraindicated in pregnancy due to its teratogenic effects and risk of agenesis of the foetal adrenals. Normal pregnancies have been reported years after the discontinuation of mitotane (8, 114).

Psychosis

The psychiatric manifestations of Cushing's are common; however, true psychosis requiring treatment is rare. Severe

depression and acute psychosis improve rapidly upon correction of hypercortisolaemia; however, less severe psychiatric symptoms may persist for several months. Medical therapy aiming for the rapid control of hypercortisolaemia may be crucial for the management of these patients, who due to their mental status may not be able to consent to definitive treatment. Metyrapone, KTZ and etomidate have been used for this indication; due to its slow-onset action, mitotane is not useful in this scenario.

In a case report of a patient with severe depression, who was lacking capacity to consent to surgery for removal of an adrenal adenoma, routine doses of metyrapone lead to a rapid improvement of the patient's mental state and after a few weeks on treatment the patient was able to consent (137). Jeffcoate *et al.* reported the presence of psychiatric symptoms in 55% of patients with CS; treatment with metyrapone improved cortisol excess and psychiatric symptoms such as depression and mood changes (6, 138). Similarly, KTZ alone (31) or in combination with metyrapone (139) has been successfully used to treat acute psychosis related with hypercortisolaemia.

Etomidate is particularly valuable in acute psychotic patients who are unable to comply with oral therapy; the parenteral administration along with the sedative effect may be the only way of managing some patients, provided they can be cared for in a Critical Care Unit. Psychotic symptoms improve, usually within days (116, 118). Sedative doses are not essential for the rapid control of hypercortisolaemia; they can be used if short-term sedation is felt to be beneficial. Mifepristone, a glucocorticoid receptor has also been shown to improve depression and cognition scores in patients with CD treated for at least 30 days (12, 140, 141).

Combination treatments

Treatment with more than one steroid inhibitor or a combination of other agents (e.g. cabergoline) and steroid inhibitors has been used in refractory cases, where rapid control is desirable and where high doses were not tolerated. Commonly, KTZ has been combined with metyrapone; however, the success of this approach depends on the sufficient dose titration of each medication. In milder disease combining metyrapone with KTZ is reasonable after only a few months of either drug used alone as monotherapy if this has not proven sufficiently effective (3, 32), but in severe cases early use of combination therapy should be considered. In the first few months of treatment with mitotane, a combination

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R274
--------	------------------------------------	--	-------	------

with one of the rapid-acting steroidogenesis inhibitors may be essential to provide early control of hypercortisolaemia until the effect of mitotane takes place.

Recently, Kamenicky *et al.* have reported the combination of high-dose metyrapone, KTZ and mitotane in 11 patients with ACTH-dependent CD and severe complications due to hypercortisolism as an alternative to emergency high-risk bilateral adrenalectomy. This regimen was used for up to 9 months and it was well tolerated. All patients had rapid clinical improvement and normalisation of UFC, and five patients were eventually able to undergo surgery on the primary tumour after a few months of treatment (142).

Dosing

Steroidogenesis inhibitors are either used in a dose titration or a block-and-replace regimen. In dose titration, a dose of the medication is initiated and then up-titrated according to the biochemical response. With block and replace, the aim is to completely abolish cortisol production and replace glucocorticoids to avoid hypoadrenalism: usually, a higher dose is initiated and quickly up-titrated to the maximum tolerated dose and replacement with glucocorticoid. The two treatment regimens have not been compared and the choice broadly depends on clinician experience and preference, but if there is evidence of cyclical disease block-and-replace regimens may be preferable. In cases where hypercortisolaemia is very severe and unlikely to respond to low doses of oral medication, a block-and-replace regimen may allow rapid and safe control of hypercortisolism with a high dose without multiple interim biochemical checks. The only risk is that incompletely treated and persistent hypercortisolaemia is made worst by the unnecessary addition of exogenous glucocorticoids.

In a dose titration regimen, KTZ is usually initiated at 400–600 mg daily in divided doses (Table 3). The dose then is up-titrated every 3–7 days according to the biochemical response to a maximum dose of 1600 mg daily. Doses above 1200 mg are less well tolerated (Table 3).

Metyrapone is usually initiated at low doses (500–750 mg/day) in benign adrenal disease, but higher doses (1500–3000 mg) in severe hypercortisolaemia, especially if due to ACTH-dependent disease or ACC. Overall, usual doses for metyrapone are 0.5–6 g daily; however, high doses may not be well tolerated and biochemical control is usually achieved with doses around 1500 mg daily in most patients (6). Due to its short half-life, the total daily dose should be divided into 3–4 smaller doses. The metabolism

of metyrapone is reduced by oestrogens and accelerated by phenytoin and phenobarbital; therefore, the dose should be adjusted appropriately in concurrent treatment and inadequate control of hypercortisolaemia.

The starting dose of mitotane is usually 1.5–6 g, in divided doses, and it is gradually up-titrated according to response and tolerability. When used as an adjuvant therapy in adrenocortical carcinoma with residual disease, the response to treatment can be assessed using radiological imaging. Patients are more likely to respond to the adrenolytic effect when plasma levels are above 14 mg/l. This is important for patients with adrenocortical carcinoma (77, 143, 144). Levels above 20 mg/l increase the risk of neurotoxicity and should be avoided. For the anti-steroidogenic effect, lower levels are likely to be effective; therefore, in hypercortisolaemia we are aiming for levels above 8.5 mg/l (8). Mitotane has poor bioavailability following oral administration (40%), and it is therefore possible that a significant number of treated patients may not achieve therapeutic levels for several months following the onset of treatment (145).

Mitotane alters the metabolism of glucocorticoids and therefore patients on a block-and-replacement regimen will need an increased dose of replacement steroids once it is established that a full blockade is achieved (57, 80). Hypoadrenalism can occur with normal replacement doses (66, 146, 147). The metabolism of dexamethasone is particularly affected and should be avoided in these patients. Hydrocortisone is a better choice and the dose given should be at least double the normal replacement dose. Close monitoring is essential, as some patients may need considerably higher doses of glucocorticoid replacement (66).

Monitoring

Monitoring of the biochemical response to treatment with a steroidogenesis inhibitor is essential, not only for titrating and assessing the dose response appropriately, but also for diagnosing potential adrenal insufficiency as a result of overtreatment. There are many ways of assessing cortisol levels, but a 24-h collection of urine for the estimation of free cortisol (UFC) is the most commonly used method. This is a simple test to perform; however, it has some limitations such as being troublesome for patients, the inaccuracy associated with incomplete collections and the need for multiple collections. More importantly, hypoadrenalism can be missed by this method. Alternatively, early morning serum cortisol or a serum cortisol day-curve (4–6 serum cortisol measurements during a 8–5-h period) can be used. The later method is commonly used in the UK and a mean value from

a serum cortisol day-curve in the range of 150–300 nmol/l, with an assay specific for cortisol, has been shown to correlate well with the normal cortisol secretion rate as calculated by an isotopic dilution technique (13, 148).

A variety of laboratory techniques are used for measuring cortisol concentration. Immunoassays are the most commonly used; however, a number of commercial cortisol immunoassays exhibit significant cross-reactivity with cortisol precursors that may be elevated in patients treated with a steroidogenesis inhibitor. This is particularly important for metyrapone (and LCI699), which is a potent inhibitor of 11 β -hydroxylase and is known to significantly increase 11-deoxycortisol. This cortisol precursor is structurally similar to cortisol and has been shown to interfere with many commercial cortisol immunoassays leading to the overestimation of cortisol levels and the risk of patient overtreatment (149, 150). It is therefore important that patients on metyrapone have biochemical monitoring of the cortisol response using more specific laboratory methods such as mass spectrometry (151).

Etomidate is always given as a continuous i.v. infusion. A dose titration or a block-and-replace approach can be used with daily monitoring of the serum cortisol levels. In a dose titration regimen, the infusion rate should be titrated to an appropriate serum cortisol level, bearing in mind that patients on this treatment are usually unwell and stressed (9). Alternatively, with a block-and-replace approach, the patient receives etomidate and replacement i.v. steroid infusion or intramuscular steroids. Common side effects are pain at the injection site, nausea and vomiting.

Biochemical monitoring in mitotane therapy is challenging. It causes an increase in many binding globulins, including cortisol-binding globulin (CBG), which results in elevated plasma cortisol levels (152, 153, 154). For this reason, total serum cortisol level cannot be used to monitor the response to treatment or development of hypoadrenalism (152). In hypercortisolism, the response should therefore be monitored with UFC. However, UFC cannot detect hypoadrenalism, which is probably an unrecognised factor adding to this drug's limited tolerability.

The increased requirement of glucocorticoids during mitotane therapy is probably affected by the increase in CBG and hence the low serum free cortisol. For this reason, serum free (or potentially salivary) cortisol may be useful in assessing glucocorticoid sufficiency in patients on a block-and-replace regimen (154). Through the induction of CYP3A4, an important drug-metabolising microsomal monooxygenase, mitotane may interact with

various drugs including commonly prescribed anti-hypertensives, antibiotics (macrolides) and some statins (66, 147).

Patients on steroidogenesis inhibitors should have regular clinical reviews by an experienced endocrinologist to assess the clinical response to treatment, the development of adverse events or symptoms indicating adrenal insufficiency.

Conclusions

Steroidogenesis enzyme inhibitors used in CS are a diverse class of drugs with differences in their mode of action but with all achieving the same principal outcome: the reduction in cortisol synthesised by the adrenal cortex. Most of the medications currently in clinical use have been available for many decades and there is significant confidence in specialist endocrine centres (where these drugs should be used) in how they should be used. Large-scale recent retrospective studies have suggested that more active up-titration is needed than in current common clinical practice; however, adverse events and poor tolerability can be common with these agents. Retrospective analyses have limitations and efficacy of therapy and incidence of side effects are more likely to be disclosed by prospective studies. Improvements in monitoring response are also needed, and although not yet formally assessed, salivary cortisol holds promise for this purpose. As Temple and Liddle reported more than 40 years ago, 'as long as there are people who might benefit by reduction of their levels of mineralocorticoids, glucocorticoids, androgens or oestrogens, there will be at least a theoretical need for safe, effective, selective inhibitors of steroid biosynthesis' (4).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

J D C Newell-Price has received consultancy fees and research support from HRA Pharma and Novartis. E Daniel has received research support from HRA Pharma and 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)

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R276
--------	------------------------------------	--	-------	------

- 2 Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ *et al.* 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)
- 3 Starkman MN, Schteingart DE & Schork MA. Cushing's syndrome after treatment: changes in cortisol and ACTH levels, and amelioration of the depressive syndrome. *Psychiatry Research* 1986 **19** 177–188. (doi:10.1016/0165-1781(86)90096-X)
- 4 Temple TE & Liddle GW. Inhibitors of adrenal steroid biosynthesis. *Annual Review of Pharmacology* 1970 **10** 199–218. (doi:10.1146/annurev.pa.10.040170.001215)
- 5 Castinetti F, Guignat L, Giraud P, Muller M, Kamenicky P, Drui D, Caron P, Luca F, Donadille B, Vantyghem MC *et al.* Ketoconazole in Cushing's disease: is it worth a try? *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1623–1630. (doi:10.1210/jc.2013-3628)
- 6 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)
- 7 Daniel E, Aylwin S, Mustafa O, Ball S, Munir A, Boelaert K, Chortis V, Cuthbertson DJ, Daousi C, Rajeev S *et al.* Effectiveness of metyrapone in 195 patients with Cushing's syndrome. *Endocrine Abstracts* 2014 **34** OC6.5. (doi:10.1530/endoabs.34.OC6.5)
- 8 Baudry C, Coste J, Khalil RB, Silvera S, Guignat L, Guibourdenche J, Abbas H, Legmann P, Bertagna X & Bertherat J. Efficiency and tolerance of mitotane in Cushing's disease in 76 patients from a single center. *European Journal of Endocrinology* 2012 **167** 473–481. (doi:10.1530/EJE-12-0358)
- 9 Preda VA, Sen J, Karavitaki N & Grossman AB. Etomidate in the management of hypercortisolaemia in Cushing's syndrome: a review. *European Journal of Endocrinology* 2012 **167** 137–143. (doi:10.1530/EJE-12-0746)
- 10 Bertagna X, Pivonello R, Fleseriu M, Zhang Y, Robinson P, Taylor A, Watson CE, Maldonado M, Hamrahian AH, Boscaro M *et al.* LCI699, a potent 11 β -hydroxylase inhibitor, normalizes urinary cortisol in patients with Cushing's disease: results from a multicenter, proof-of-concept study. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1375–1383. (doi:10.1210/jc.2013-2117)
- 11 Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, Schoenherr U, Mills D, Salgado LR, Biller BM *et al.* 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)
- 12 Fleseriu M, Biller BM, Findling JW, Molitch ME, Schteingart DE, Gross C & Investigators SS. Mifepristone, a glucocorticoid receptor antagonist, produces clinical and metabolic benefits in patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 2039–2049. (doi:10.1210/jc.2011-3350)
- 13 Trainer PJ. Next generation medical therapy for Cushing's syndrome – can we measure a benefit? *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1157–1160. (doi:10.1210/jc.2014-1054)
- 14 Rosenblatt HM, Byrne W, Ament ME, Graybill J & Stiehm ER. Successful treatment of chronic mucocutaneous candidiasis with ketoconazole. *Journal of Pediatrics* 1980 **97** 657–660. (doi:10.1016/S0022-3476(80)80036-9)
- 15 Kirkpatrick CH, Petersen EA & Alling DW. Treatment of chronic mucocutaneous candidosis with ketoconazole – preliminary results of a controlled, double-blind clinical trial. *Reviews of Infectious Diseases* 1980 **2** 599. (doi:10.1093/clinids/2.4.599)
- 16 Symoens J, Moens M, Dom J, Scheijgrond H, Dony J, Schuermans V, Legendre R & Finestine N. An evaluation of 2 years of clinical-experience with ketoconazole. *Reviews of Infectious Diseases* 1980 **2** 674–691. (doi:10.1093/clinids/2.4.674)
- 17 Heeres J, Back LJ, Mostmans JH & Vancutsem J. Anti-mycotic imidazoles. Part .4. Synthesis and antifungal activity of ketoconazole, a new potent orally active broad-spectrum antifungal agent. *Journal of Medicinal Chemistry* 1979 **22** 1003–1005. (doi:10.1021/jm00194a023)
- 18 DeFelice R, Johnson DG & Galgiani JN. Gynecomastia with ketoconazole. *Antimicrobial Agents and Chemotherapy* 1981 **19** 1073–1074. (doi:10.1128/AAC.19.6.1073)
- 19 Pont A, Williams PL, Azhar S, Reitz RE, Bochra C, Smith ER & Stevens DA. Ketoconazole blocks testosterone synthesis. *Archives of Internal Medicine* 1982 **142** 2137–2140. (doi:10.1001/archinte.1982.00340250097015)
- 20 Santen RJ, Van den Bossche H, Symoens J, Brugmans J & DeCoster R. Site of action of low dose ketoconazole on androgen biosynthesis in men. *Journal of Clinical Endocrinology and Metabolism* 1983 **57** 732–736. (doi:10.1210/jcem-57-4-732)
- 21 Pont A, Williams PL, Loose DS, Feldman D, Reitz RE, Bochra C & Stevens DA. Ketoconazole blocks adrenal steroid synthesis. *Annals of Internal Medicine* 1982 **97** 370–372. (doi:10.7326/0003-4819-97-3-370)
- 22 Loose DS, Kan PB, Hirst MA, Marcus RA & Feldman D. Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes. *Journal of Clinical Investigation* 1983 **71** 1495–1499. (doi:10.1172/JCI110903)
- 23 Kowal J. The effect of ketoconazole on steroidogenesis in cultured mouse adrenal cortex tumor cells. *Endocrinology* 1983 **112** 1541–1543. (doi:10.1210/endo-112-4-1541)
- 24 Mortimer RH, Cannell GR, Thew CM & Galligan JP. Ketoconazole and plasma and urine steroid levels in Cushing's disease. *Clinical and Experimental Pharmacology & Physiology* 1991 **18** 563–569. (doi:10.1111/j.1440-1681.1991.tb01492.x)
- 25 Engelhardt D, Weber MM, Miksch T, Abedinpour F & Jaspers C. The influence of ketoconazole on human adrenal steroidogenesis: incubation studies with tissue slices. *Clinical Endocrinology* 1991 **35** 163–168. (doi:10.1111/j.1365-2265.1991.tb03516.x)
- 26 Feldman D. Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. *Endocrine Reviews* 1986 **7** 409–420. (doi:10.1210/edrv-7-4-409)
- 27 Blum RA, D'Andrea DT, Florentino BM, Wilton JH, Hilligoss DM, Gardner MJ, Henry EB, Goldstein H & Schentag JJ. Increased gastric pH and the bioavailability of fluconazole and ketoconazole. *Annals of Internal Medicine* 1991 **114** 755–757. (doi:10.7326/0003-4819-114-9-755)
- 28 Gal M & Orly J. Selective inhibition of steroidogenic enzymes by ketoconazole in rat ovary cells. *Clinical Medicine Insights. Reproductive Health* 2014 **8** 15–22. (doi:10.4137/CMRH.S14036)
- 29 Gal M, Barr I, Lior O, Tadmor O, Orly J & Diamant YZ. Effect of ketoconazole on steroidogenic human granulosa–luteal cells in culture. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 1991 **39** 209–214. (doi:10.1016/0028-2243(91)90059-T)
- 30 Sonino N, Boscaro M, Merola G & Mantero F. Prolonged treatment of Cushing's disease by ketoconazole. *Journal of Clinical Endocrinology and Metabolism* 1985 **61** 718–722. (doi:10.1210/jcem-61-4-718)
- 31 Sonino N, Boscaro M, Paoletta A, Mantero F & Ziliotto D. Ketoconazole treatment in Cushing's syndrome: experience in 34 patients. *Clinical Endocrinology* 1991 **35** 347–352. (doi:10.1111/j.1365-2265.1991.tb03547.x)
- 32 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)
- 33 McCance DR, Ritchie CM, Sheridan B & Atkinson AB. Acute hypoadrenalism and hepatotoxicity after treatment with ketoconazole. *Lancet* 1987 **1** 573. (doi:10.1016/S0140-6736(87)90222-4)
- 34 Angeli A & Frairia R. Ketoconazole therapy in Cushing's disease. *Lancet* 1985 **1** 821. (doi:10.1016/S0140-6736(85)91482-5)
- 35 Loli P, Berselli ME & Tagliaferri M. Use of ketoconazole in the treatment of Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1986 **63** 1365–1371. (doi:10.1210/jcem-63-6-1365)
- 36 Van Der Meer JW, Keuning JJ, Scheijgrond HW, Heykants J, Van Cutsem J & Brugmans J. The influence of gastric acidity on the

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R277
--------	------------------------------------	--	-------	------

- bio-availability of ketoconazole. *Journal of Antimicrobial Chemotherapy* 1980 **6** 552–554. (doi:10.1093/jac/6.4.552)
- 37 Chin TW, Loeb M & Fong IW. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrobial Agents and Chemotherapy* 1995 **39** 1671–1675. (doi:10.1128/AAC.39.8.1671)
 - 38 Agency EM. Suspension of marketing authorisations for oral ketoconazole. Benefit of oral ketoconazole does not outweigh risk of liver injury in fungal infections, 2013. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Ketoconazole-containing_medicines/human_referral_000348.jsp&mid=WC0b01ac05805c516f
 - 39 FDA. FDA Drug Safety Communication: FDA limits usage of Nizoral (ketoconazole) oral tablets due to potentially fatal liver injury and risk of drug interactions and adrenal gland problems, 2013. <http://www.fda.gov/Drugs/DrugSafety/ucm362415.htm>
 - 40 Lake-Bakaar GSP & Sherlock S. Hepatic reactions associated with ketoconazole in the United Kingdom. *BMJ* 1987 **294** 419–422. (doi:10.1136/bmj.294.6569.419)
 - 41 Heiberg JK & Svejgaard E. Toxic hepatitis during ketoconazole treatment. *BMJ* 1981 **283** 825–826. (doi:10.1136/bmj.283.6295.825)
 - 42 Bercoff E, Bernuau J, Degott C, Kalis B, Lemaire A, Tilly H, Rueff B & Benhamou JP. Ketoconazole-induced fulminant hepatitis. *Gut* 1985 **26** 636–638. (doi:10.1136/gut.26.6.636)
 - 43 Newell-Price J. Ketoconazole as an adrenal steroidogenesis inhibitor: effectiveness and risks in the treatment of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1586–1588. (doi:10.1210/jc.2014-1622)
 - 44 Liddle GW, Island D, Lance EM & Harris AP. Alterations of adrenal steroid patterns in man resulting from treatment with a chemical inhibitor of 11 β -hydroxylation. *Journal of Clinical Endocrinology and Metabolism* 1958 **18** 906–912. (doi:10.1210/jcem-18-8-906)
 - 45 Rigel DF, Fu F, Beil M, Hu CW, Liang G & Jeng AY. Pharmacodynamic and pharmacokinetic characterization of the aldosterone synthase inhibitor FAD286 in two rodent models of hyperaldosteronism: comparison with the 11 β -hydroxylase inhibitor metyrapone. *Journal of Pharmacology and Experimental Therapeutics* 2010 **334** 232–243. (doi:10.1124/jpet.110.167148)
 - 46 Coppage WS Jr, Island D, Smith M & Liddle GW. Inhibition of aldosterone secretion and modification of electrolyte excretion in man by a chemical inhibitor of 11 β -hydroxylation. *Journal of Clinical Investigation* 1959 **38** 2101–2110. (doi:10.1172/JCI103988)
 - 47 Sonino N, Chow D, Levine LS & New MI. Clinical response to metyrapone as indicated by measurement of mineralocorticoids and glucocorticoids in normal children. *Clinical Endocrinology* 1981 **14** 31–39. (doi:10.1111/j.1365-2265.1981.tb00362.x)
 - 48 Strott CA, West CD, Nakagawa K, Kondo T & Tyler FH. Plasma 11-deoxycorticosteroid and ACTH response to metyrapone (plasma metyrapone test). *Journal of Clinical Endocrinology and Metabolism* 1969 **29** 6–11. (doi:10.1210/jcem-29-1-6)
 - 49 Liddle GW, Estep HL, Kendall JW Jr, Williams WC Jr & Townes AW. Clinical application of a new test of pituitary reserve. *Journal of Clinical Endocrinology and Metabolism* 1959 **19** 875–894. (doi:10.1210/jcem-19-8-875)
 - 50 Spiger M, Jubiz W, Meikle AW, West CD & Tylor FH. Single-dose metyrapone test: review of a four-year experience. *Archives of Internal Medicine* 1975 **135** 698–700. (doi:10.1001/archinte.1975.00330050072012)
 - 51 Child DF, Burke CW, Burley DM, Rees LH & Fraser TR. Drug controlled of Cushing's syndrome. Combined aminoglutethimide and metyrapone therapy. *Acta Endocrinologica* 1976 **82** 330–341. (doi:10.1530/acta.0.0820330)
 - 52 Jeffcoate WJ, Rees LH, Tomlin S, Jones AE, Edwards CR & Besser GM. Metyrapone in long-term management of Cushing's disease. *BMJ* 1977 **2** 215–217. (doi:10.1136/bmj.2.6081.215)
 - 53 Ross WM, Evered DC, Hunter P, Benaim M, Cook D & Hall R. Treatment of Cushing's disease with adrenal blocking drugs and megavoltage therapy to the pituitary. *Clinical Radiology* 1979 **30** 149–153. (doi:10.1016/S0009-9260(79)80135-X)
 - 54 Storr HL, Plowman PN, Carroll PV, François I, Krassas GE, Afshar F, Besser GM, Grossman AB & Savage MO. Clinical and endocrine responses to pituitary radiotherapy in pediatric Cushing's disease: an effective second-line treatment. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 34–37. (doi:10.1210/jc.2002-021032)
 - 55 Storr HL, Mitchell H, Swords FM, Main KM, Hindmarsh PC, Betts PR, Shaw NJ, Johnston DI, Clark AJ, Reznick RH *et al.* Clinical features, diagnosis, treatment and molecular studies in paediatric Cushing's syndrome due to primary nodular adrenocortical hyperplasia. *Clinical Endocrinology* 2004 **61** 553–559. (doi:10.1111/j.1365-2265.2004.02124.x)
 - 56 Blevins LS, Christy JH, Khajavi M & Tindall GT. Outcomes of therapy for Cushing's disease due to adrenocorticotropin-secreting pituitary macroadenomas. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 63–67. (doi:10.1210/jcem.83.1.4525)
 - 57 Cobey FA, Taliaferro I & Haag HB. Effect of DDD and some of its derivatives on plasma 17-OH-corticosteroids in the dog. *Science* 1956 **123** 140–141. (doi:10.1126/science.123.3187.140)
 - 58 Cueto C, Brown JH & Richardson AP Jr. Biological studies on an adrenocorticolytic agent and the isolation of the active components. *Endocrinology* 1958 **62** 334–339. (doi:10.1210/endo-62-3-334)
 - 59 Bergenstal DM, Hertz R, Lipsett MB & Mays RH. Chemotherapy of adrenocortical cancer with o,p'DDD. *Annals of Internal Medicine* 1960 **53** 672–682. (doi:10.7326/0003-4819-53-4-672)
 - 60 Nelson AA & Woodard G. Severe adrenal cortical atrophy (cytotoxic) and hepatic damage produced in dogs by feeding 2,2-bis(parachlorophenyl)-1,1-dichloroethane (DDD or TDE). *Archives of Pathology* 1949 **48** 387–394.
 - 61 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)
 - 62 Luton JP, Mahoudeau JA, Bouchard P, Thieblot P, Hauteceuvre 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)
 - 63 Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M, Penteroudakis G & Group EGW. Adrenal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology* 2012 **23** (Suppl 7) vii131–vii138. (doi:10.1093/annonc/mds231)
 - 64 Hart MM, Swackhamer ES & Straw JA. Studies on the site of action of o,p'-DDD in the dog adrenal cortex. II. TPNH- and corticosteroid precursor-stimulation of o,p'-DDD inhibited steroidogenesis. *Steroids* 1971 **17** 575–586. (doi:10.1016/S0039-128X(71)80158-7)
 - 65 Brown RD, Nicholson WE, Chick WT & Strott CA. Effect of o,p'DDD on human adrenal steroid 11 β -hydroxylation activity. *Journal of Clinical Endocrinology and Metabolism* 1973 **36** 730–733. (doi:10.1210/jcem-36-4-730)
 - 66 Chortis V, Taylor AE, Schneider P, Tomlinson JW, Hughes BA, O'Neil DM, Libe R, Allolio B, Bertagna X, Bertherat J *et al.* Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5 α -reductase, explaining the need for personalized glucocorticoid and androgen replacement. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 161–171. (doi:10.1210/jc.2012-2851)
 - 67 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. (doi:10.7326/0003-4819-92-5-613)
 - 68 Daffara F, De Francia S, Reimondo G, Zaggia B, Aroasio E, Porpiglia F, Volante M, Termine A, Di Carlo F, Dogliotti L *et al.* Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. *Endocrine-Related Cancer* 2008 **15** 1043–1053. (doi:10.1677/ERC-08-0103)

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R278
--------	------------------------------------	--	-------	------

- 69 Kaminsky N, Luse S & Hartroft P. Ultrastructure of adrenal cortex of the dog during treatment with DDD. *Journal of the National Cancer Institute* 1962 **29** 127–159.
- 70 Schteingart DE, Sinsheimer JE, Counsell RE, Abrams GD, McClellan N, Djanegara T, Hines J, Ruangwises N, Benitez R & Wotring LL. Comparison of the adrenalytic activity of mitotane and a methylated homolog on normal adrenal cortex and adrenal cortical carcinoma. *Cancer Chemotherapy and Pharmacology* 1993 **31** 459–466. (doi:10.1007/BF00685036)
- 71 Poli G, Guasti D, Rapizzi E, Fucci R, Canu L, Bandini A, Cini N, Bani D, Mannelli M & Luconi M. Morphofunctional effects of mitotane on mitochondria in human adrenocortical cancer cells. *Endocrine-Related Cancer* 2013 **20** 537–550. (doi:10.1530/ERC-13-0150)
- 72 Schteingart DE, Doherty GM, Gauger PG, Giordano TJ, Hammer GD, Korobkin M & Worden FP. Management of patients with adrenal cancer: recommendations of an international consensus conference. *Endocrine-Related Cancer* 2005 **12** 667–680. (doi:10.1677/erc.1.01029)
- 73 Cueto C & Brown JH. The chemical fractionation of an adrenocortically drug. *Endocrinology* 1958 **62** 326–333. (doi:10.1210/endo-62-3-326)
- 74 Lindhe Ö, Skogseid B & Brandt I. Cytochrome P450-catalyzed binding of 3-methylsulfonyl-DDE and o,p'-DDD in human adrenal zona fasciculata/reticularis. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1319–1326. (doi:10.1210/jcem.87.3.8281)
- 75 Nieman LK. Medical therapy of Cushing's disease. *Pituitary* 2002 **5** 77–82. (doi:10.1023/A:1022308429992)
- 76 Toutou Y, Moolenaar AJ, Bogdan A, Auzéby A & Luton JP. o,p'-DDD (mitotane) treatment for Cushing's syndrome: adrenal drug concentration and inhibition *in vitro* of steroid synthesis. *European Journal of Clinical Pharmacology* 1985 **29** 483–487. (doi:10.1007/BF00613466)
- 77 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)
- 78 Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E *et al.* Adjuvant mitotane treatment for adrenocortical carcinoma. *New England Journal of Medicine* 2007 **356** 2372–2380. (doi:10.1056/NEJMoa063360)
- 79 Godefroi EF, Janssen PAJ, Vanderey CA, Vanheert AH & Niemegeer CJ. DI-1-(1-arylalkyl)imidazole-5-carboxylate esters. A novel type of hypnotic agents. *Journal of Medicinal Chemistry* 1965 **8** 220–223. (doi:10.1021/jm00326a017)
- 80 Fellows IW, Byrne AJ & Allison SP. Adrenocortical suppression with etomidate. *Lancet* 1983 **2** 54–55. (doi:10.1016/S0140-6736(83)90043-0)
- 81 Ledingham IM & Watt I. Influence of sedation on mortality in critically ill multiple trauma patients. *Lancet* 1983 **1** 1270. (doi:10.1016/S0140-6736(83)92712-5)
- 82 Chee HD, Bronsveld W, Lips PTAM & Thijs LG. Adrenocortical suppression in multiply injured patients: a complication of etomidate treatment. *BMJ* 1984 **288** 485–486. (doi:10.1136/bmj.288.6415.485-b)
- 83 Watt I & Ledingham IM. Mortality amongst multiple trauma patients admitted to an intensive therapy unit. *Anaesthesia* 1984 **39** 973–981. (doi:10.1111/j.1365-2044.1984.tb08885.x)
- 84 Lambert A, Mitchell R, Frost J, Ratcliffe JG & Robertson WR. Direct *in vitro* inhibition of adrenal steroidogenesis by etomidate. *Lancet* 1983 **2** 1085–1086. (doi:10.1016/S0140-6736(83)91070-X)
- 85 de Jong FH, Mallios C, Jansen C, Scheck PA & Lamberts SW. Etomidate suppresses adrenocortical function by inhibition of 11 β -hydroxylation. *Journal of Clinical Endocrinology and Metabolism* 1984 **59** 1143–1147. (doi:10.1210/jcem-59-6-1143)
- 86 Allolio B, Stuttmann R, Leonhard U, Fischer H & Winkelmann W. Adrenocortical suppression by a single induction dose of etomidate. *Klinische Wochenschrift* 1984 **62** 1014–1017. (doi:10.1007/BF01711723)
- 87 Flynn G & Shehabi Y. Pro/con debate: is etomidate safe in hemodynamically unstable critically ill patients? *Critical Care* 2012 **16** 227. (doi:10.1186/cc11242)
- 88 Griesdale DE. Etomidate for intubation of patients who have sepsis or septic shock – where do we go from here? *Critical Care* 2012 **16** 189. (doi:10.1186/cc11889)
- 89 Budde AO & Mets B. Pro: etomidate is the ideal induction agent for a cardiac anesthetic. *Journal of Cardiothoracic and Vascular Anesthesia* 2013 **27** 180–183. (doi:10.1053/j.jvca.2012.08.024)
- 90 Malhotra AK. Con: etomidate – the ideal induction agent for a cardiac anesthetic? *Journal of Cardiothoracic and Vascular Anesthesia* 2013 **27** 178–179. (doi:10.1053/j.jvca.2012.08.023)
- 91 Allolio B, Dorr H, Stuttmann R, Knorr D, Engelhardt D & Winkelmann W. Effect of a single bolus of etomidate upon eight major corticosteroid hormones and plasma ACTH. *Clinical Endocrinology* 1985 **22** 281–286. (doi:10.1111/j.1365-2265.1985.tb03241.x)
- 92 Schulte HM, Benker G, Reinwein D, Sippell WG & Allolio B. Infusion of low dose etomidate: correction of hypercortisolemia in patients with Cushing's syndrome and dose-response relationship in normal subjects. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 1426–1430. (doi:10.1210/jcem-70-5-1426)
- 93 Diago MC, Amado JA, Otero M & Lopez-Cordova JJ. Anti-adrenal action of a subanaesthetic dose of etomidate. *Anaesthesia* 1988 **43** 644–645. (doi:10.1111/j.1365-2044.1988.tb04148.x)
- 94 Absalom A, Pledger D & Kong A. Adrenocortical function in critically ill patients 24 h after a single dose of etomidate. *Anaesthesia* 1999 **54** 861–867. (doi:10.1046/j.1365-2044.1999.01003.x)
- 95 Drake WM, Perry LA, Hinds CJ, Lowe DG, Reznick RH & Besser GM. Emergency and prolonged use of intravenous etomidate to control hypercortisolemia in a patient with Cushing's syndrome and peritonitis. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3542–3544. (doi:10.1210/jcem.83.10.5156)
- 96 Krakoff J, Koch CA, Calis KA, Alexander RH & Nieman LK. Use of a parenteral propylene glycol-containing etomidate preparation for the long-term management of ectopic Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 4104–4108. (doi:10.1210/jcem.86.9.7870)
- 97 Varga I, Racz K, Kiss R, Futo L, Toth M, Sergev O & Glaz E. Direct inhibitory effect of etomidate on corticosteroid secretion in human pathological adrenocortical cells. *Steroids* 1993 **58** 64–68. (doi:10.1016/0039-128X(93)90054-Q)
- 98 Allolio B, Schulte HM, Kaulen D, Reincke M, Jaurisch-Hancke C & Winkelmann W. Nonhypnotic low-dose etomidate for rapid correction of hypercortisolemia in Cushing's syndrome. *Klinische Wochenschrift* 1988 **66** 361–364. (doi:10.1007/BF01735795)
- 99 Soh LM, Gunganah K, Akker SA, Jones P, Khachi H, Dodzo K & Drake WM. Etomidate in the emergency management of hypercortisolemia. *European Journal of Endocrinology* 2012 **167** 727–728; author reply 729. (doi:10.1530/EJE-12-0698)
- 100 Forman SA. Clinical and molecular pharmacology of etomidate. *Anesthesiology* 2011 **114** 695–707. (doi:10.1097/ALN.0b013e3181ff72b5)
- 101 WHO. Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives. In Wld Hlth Org. techn. Rep. Ser., 1974, No. 539; Geneva: WHO, 1973. <http://www.inchem.org/documents/jecfa/jecmono/v05je94.htm>
- 102 Johnson TN & Canada TW. Etomidate use for Cushing's syndrome caused by an ectopic adrenocorticotrophic hormone-producing tumor. *Annals of Pharmacotherapy* 2007 **41** 350–353. (doi:10.1345/aph.1H365)
- 103 Wilson KC, Reardon C, Theodore AC & Farber HW. Propylene glycol toxicity: a severe iatrogenic illness in ICU patients receiving IV benzodiazepines: a case series and prospective, observational pilot study. *Chest* 2005 **128** 1674–1681. (doi:10.1378/chest.128.3.1674)
- 104 Calhoun DA, White WB, Krum H, Guo WN, Bermann G, Trapani A, Lefkowitz MP & Menard J. Effects of a novel aldosterone synthase inhibitor for treatment of primary hypertension results of a

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R279
--------	------------------------------------	--	-------	------

- randomized, double-blind, placebo- and active-controlled phase 2 trial. *Circulation* 2011 **124** 1945–1955. (doi:10.1161/CIRCULATIONAHA.111.029892)
- 105 Amar L, Azizi M, Menard J, Peyrard S, Watson C & Plouin PF. Aldosterone synthase inhibition with LCI699 a proof-of-concept study in patients with primary aldosteronism. *Hypertension* 2010 **56** 831–838. (doi:10.1161/HYPERTENSIONAHA.110.157271)
 - 106 Karns AD, Bral JM, Hartman D, Peppard T & Schumacher C. Study of aldosterone synthase inhibition as an add-on therapy in resistant hypertension. *Journal of Clinical Hypertension* 2013 **15** 186–192. (doi:10.1111/jch.12051)
 - 107 Beverly Biller JY, Hamrahian A, Fleseriu M, Molitch M, Pivonello R, Shimatsu A, Shimizu C, Tanaka T, White T, Hilliard A *et al*. Normalization of urinary cortisol with the potent 11 β -hydroxylase inhibitor LCI699 in patients with Cushing's disease: 22-week, multicentre, open-label study. *Endocrine Abstracts* 2014 **35** OC8.4. (doi:10.1530/endoabs.35.OC8.4)
 - 108 Menard Joël WC, Rebello S, Zhang Yiming M & Dole William P. Hormonal and electrolyte responses to the aldosterone synthetase inhibitor LCI699 in sodium depleted healthy subjects. *Journal of the American College of Cardiology* 2010 **55** A61.E583.
 - 109 Andersen K, Hartman D, Peppard T, Hermann D, Van Ess P, Lefkowitz M & Trapani A. The effects of aldosterone synthase inhibition on aldosterone and cortisol in patients with hypertension: a phase II, randomized, double-blind, placebo-controlled, multicenter study. *Journal of Clinical Hypertension* 2012 **14** 580–587. (doi:10.1111/j.1751-7176.2012.00667.x)
 - 110 Rotstein DM, Kertesz DJ, Walker KAM & Swinney DC. Stereoisomers of ketoconazole – preparation and biological-activity. *Journal of Medicinal Chemistry* 1992 **35** 2818–2825. (doi:10.1021/jm00093a015)
 - 111 Schwartz SL, Rendell M, Ahmann AJ, Thomas A, Arauz-Pacheco CJ & Welles BR. Safety profile and metabolic effects of 14 days of treatment with DIO-902: results of a phase IIa multicenter, randomized, double-blind, placebo-controlled, parallel-group trial in patients with type 2 diabetes mellitus. *Clinical Therapeutics* 2008 **30** 1081–1088. (doi:10.1016/j.clinthera.2008.05.021)
 - 112 Arakaki R & Welles B. Ketoconazole enantiomer for the treatment of diabetes mellitus. *Expert Opinion on Investigational Drugs* 2010 **19** 185–194. (doi:10.1517/13543780903381411)
 - 113 ClinicalTrials.gov. Treatment for Endogenous Cushing's Syndrome. edn 2013. <http://www.ClinicalTrials.gov>.
 - 114 Leiba S, Weinstein R, Shindel B, Lapidot M, Stern E, Levavi H, Rusecki Y & Abramovici A. The protracted effect of o,p'-DDD in Cushing's disease and its impact on adrenal morphogenesis of young human embryo. *Annales d'Endocrinologie* 1989 **50** 49–53.
 - 115 Little MD, Shalet SM, Beardwell CG, Ahmed SR & Sutton ML. Long-term follow-up of low-dose external pituitary irradiation for Cushing's disease. *Clinical Endocrinology* 1990 **33** 445–455. (doi:10.1111/j.1365-2265.1990.tb03883.x)
 - 116 Gartner R, Albrecht M & Muller OA. Effect of etomidate on hypercortisolism due to ectopic ACTH production. *Lancet* 1986 **1** 275. (doi:10.1016/S0140-6736(86)90811-1)
 - 117 Dabbagh A, Sa'adat N & Heidari Z. Etomidate infusion in the critical care setting for suppressing the acute phase of Cushing's syndrome. *Anesthesia and Analgesia* 2009 **108** 238–239. (doi:10.1213/ane.0b013e318187ed37)
 - 118 Chan LF, Vaidya M, Westphal B, Allgrove J, Martin L, Afshar F, Hindmarsh PC, Savage MO, Grossman AB & Storr HL. Use of intravenous etomidate to control acute psychosis induced by the hypercortisolaemia in severe paediatric Cushing's disease. *Hormone Research in Paediatrics* 2011 **75** 441–446. (doi:10.1159/000324419)
 - 119 Saketos M, Sharma N & Santoro NF. Suppression of the hypothalamic–pituitary–ovarian axis in normal women by glucocorticoids. *Biology of Reproduction* 1993 **49** 1270–1276. (doi:10.1095/biolreprod49.6.1270)
 - 120 Padmanabhan V, Keech C & Convey EM. Cortisol inhibits and adrenocorticotropin has no effect on luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells *in vitro*. *Endocrinology* 1983 **112** 1782–1787. (doi:10.1210/endo-112-5-1782)
 - 121 Lindsay JR, Jonklaas J, Oldfield EH & Nieman LK. Cushing's syndrome during pregnancy: personal experience and review of the literature. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3077–3083. (doi:10.1210/jc.2004-2361)
 - 122 Lekarev O & New MI. Adrenal disease in pregnancy. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2011 **25** 959–973. (doi:10.1016/j.beem.2011.08.004)
 - 123 Wallace C, Toth EL, Lewanczuk RZ & Siminoski K. Pregnancy-induced Cushing's syndrome in multiple pregnancies. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 15–21. (doi:10.1210/jcem.81.1.8550743)
 - 124 Boronat M, Marrero D, Lopez-Plasencia Y, Barber M, Schamann Y & Novoa FJ. Successful outcome of pregnancy in a patient with Cushing's disease under treatment with ketoconazole during the first trimester of gestation. *Gynecological Endocrinology* 2011 **27** 675–677. (doi:10.3109/09513590.2010.521268)
 - 125 Berwaerts J, Verhelst J, Mahler C & Abs R. Cushing's syndrome in pregnancy treated by ketoconazole: case report and review of the literature. *Gynecological Endocrinology* 1999 **13** 175–182. (doi:10.3109/09513599909167552)
 - 126 Gormley MJ, Hadden DR, Kennedy TL, Montgomery DA, Murnaghan GA & Sheridan B. Cushing's syndrome in pregnancy – treatment with metyrapone. *Clinical Endocrinology* 1982 **16** 283–293. (doi:10.1111/j.1365-2265.1982.tb00718.x)
 - 127 Schiemer R, Latibeaudiere M, Close C & Fox R. Type 2 diabetes identified in pregnancy secondary to Cushing's syndrome. *Journal of Obstetrics and Gynaecology* 2011 **31** 541. (doi:10.3109/01443615.2011.584645)
 - 128 Blanco C, Maqueda E, Rubio JA & Rodriguez A. Cushing's syndrome during pregnancy secondary to adrenal adenoma: metyrapone treatment and laparoscopic adrenalectomy. *Journal of Endocrinological Investigation* 2006 **29** 164–167. (doi:10.1007/BF03344091)
 - 129 Kasperlik-Zaluska AA, Szczupacka I, Leszczynska-Bystrzanowska J & Drus-Przybyszewska G. Pregnancy-dependent Cushing's syndrome in three pregnancies. *BJOG: an International Journal of Obstetrics and Gynaecology* 2000 **107** 810–812. (doi:10.1111/j.1471-0528.2000.tb13348.x)
 - 130 Close CF, Mann MC, Watts JF & Taylor KG. ACTH-independent Cushing's syndrome in pregnancy with spontaneous resolution after delivery: control of the hypercortisolism with metyrapone. *Clinical Endocrinology* 1993 **39** 375–379. (doi:10.1111/j.1365-2265.1993.tb02380.x)
 - 131 Cabezon C, Bruno OD, Cohen M, Garcia S & Gutman RA. Twin pregnancy in a patient with Cushing's disease. *Fertility and Sterility* 1999 **72** 371–372. (doi:10.1016/S0015-0282(99)00261-7)
 - 132 Achong N, D'Emden M, Fagermo N & Mortimer R. Pregnancy-induced Cushing's syndrome in recurrent pregnancies: case report and literature review. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 2012 **52** 96–100. (doi:10.1111/j.1479-828X.2011.01388.x)
 - 133 Shaw JA, Pearson DW, Krukowski ZH, Fisher PM & Bevan JS. Cushing's syndrome during pregnancy: curative adrenalectomy at 31 weeks gestation. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2002 **105** 189–191. (doi:10.1016/S0301-2115(02)00148-3)
 - 134 Hotham NJ, Ilett KF, Hackett LP, Morton MR, Muller P & Hague WM. Transfer of metyrapone and its metabolite, rac-metyrapol, into breast milk. *Journal of Human Lactation* 2009 **25** 451–454. (doi:10.1177/0890334409345496)
 - 135 Connell JM, Cordiner J, Davies DL, Fraser R, Frier BM & McPherson SG. Pregnancy complicated by Cushing's syndrome: potential hazard of metyrapone therapy. Case report. *British Journal of Obstetrics and Gynaecology* 1985 **92** 1192–1195. (doi:10.1111/j.1471-0528.1985.tb03037.x)
 - 136 Amado JA, Pesquera C, Gonzalez EM, Otero M, Freijanes J & Alvarez A. Successful treatment with ketoconazole of Cushing's syndrome in

Review	E Daniel and J D C Newell-Price	Steroidogenesis enzyme inhibitors in Cushing's syndrome	172:6	R280
--------	------------------------------------	--	-------	------

- pregnancy. *Postgraduate Medical Journal* 1990 **66** 221–223. (doi:10.1136/pgmj.66.773.221)
- 137 Obinata D, Yamaguchi K, Hirano D, Yoshida T, Soma M & Takahashi S. Preoperative management of Cushing's syndrome with metyrapone for severe psychiatric disturbances. *International Journal of Urology* 2008 **15** 361–362. (doi:10.1111/j.1442-2042.2008.01995.x)
 - 138 Jeffcoat WJ, Silverstone JT, Edwards CR & Besser GM. Psychiatric manifestations of Cushing's syndrome: response to lowering of plasma cortisol. *Quarterly Journal of Medicine* 1979 **48** 465–472.
 - 139 Myhill PC, Sillars BA, Starkstein S, Annus T & Yeap BB. Reduction in salivary cortisol concentration correlates with resolution of psychosis in Cushing's syndrome. *Journal of Neuropsychiatry and Clinical Neurosciences* 2008 **20** 113–115.
 - 140 Bilgin YM, van der Wiel HE, Fischer HR & De Herder WW. Treatment of severe psychosis due to ectopic Cushing's syndrome. *Journal of Endocrinological Investigation* 2007 **30** 776–779. (doi:10.1007/BF03350817)
 - 141 Chu JW, Matthias DF, Belanoff J, Schatzberg A, Hoffman AR & Feldman D. Successful long-term treatment of refractory Cushing's disease with high-dose mifepristone (RU 486). *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3568–3573. (doi:10.1210/jcem.86.8.7740)
 - 142 Kamenický P, Droumaguet C, Salenave S, Blanchard A, Jublanc C, Gautier J-F, Brailly-Tabard S, Leboulleux S, Schlumberger M, Baudin E *et al.* 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)
 - 143 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 & Clinical Oncology* 1984 **20** 47–53. (doi:10.1016/0277-5379(84)90033-6)
 - 144 Hermesen IG, Fassnacht M, Terzolo M, Houterman S, den Hartigh J, Leboulleux S, Daffara F, Berruti A, Chadarevian R, Schlumberger M *et al.* Plasma concentrations of o,p'-DDD, o,p'-DDA, and o,p'-DDE as predictors of tumor response to mitotane in adrenocortical carcinoma: results of a retrospective ENS@T multicenter study. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 1844–1851. (doi:10.1210/jc.2010-2676)
 - 145 Mauclere-Denost S, Leboulleux S, Borget I, Paci A, Young J, Al Ghuzlan A, Deandreis D, Drouard L, Tabarin A, Chanson P *et al.* High-dose mitotane strategy in adrenocortical carcinoma: prospective analysis of plasma mitotane measurement during the first 3 months of follow-up. *European Journal of Endocrinology* 2012 **166** 261–268. (doi:10.1530/EJE-11-0557)
 - 146 Robinson BG, Hales IB, Henniker AJ, Ho K, Luttrell BM, Smee IR & Stiel JN. The effect of o,p'-DDD on adrenal steroid replacement therapy requirements. *Clinical Endocrinology* 1987 **27** 437–444. (doi:10.1111/j.1365-2265.1987.tb01171.x)
 - 147 Kroiss M, Quinkler M, Lutz WK, Allolio B & Fassnacht M. Drug interactions with mitotane by induction of CYP3A4 metabolism in the clinical management of adrenocortical carcinoma. *Clinical Endocrinology* 2011 **75** 585–591. (doi:10.1111/j.1365-2265.2011.04214.x)
 - 148 Trainer PJ, Eastment C, Grossman AB, Wheeler MJ, Perry L & Besser GM. The relationship between cortisol production rate and serial serum cortisol estimation in patients on medical therapy for Cushing's syndrome. *Clinical Endocrinology* 1993 **39** 441–443. (doi:10.1111/j.1365-2265.1993.tb02391.x)
 - 149 Owen LJ, Halsall DJ & Keevil BG. Cortisol measurement in patients receiving metyrapone therapy. *Annals of Clinical Biochemistry* 2010 **47** 573–575. (doi:10.1258/acb.2010.010167)
 - 150 Monaghan PJ, Owen LJ, Trainer PJ, Brabant G, Keevil BG & Darby D. Comparison of serum cortisol measurement by immunoassay and liquid chromatography-tandem mass spectrometry in patients receiving the 11 β -hydroxylase inhibitor metyrapone. *Annals of Clinical Biochemistry* 2011 **48** 441–446. (doi:10.1258/acb.2011.011014)
 - 151 Monaghan PJ, Keevil BG & Trainer PJ. The use of mass spectrometry to improve the diagnosis and the management of the HPA axis. *Reviews in Endocrine & Metabolic Disorders* 2013 **14** 143–157. (doi:10.1007/s11154-013-9240-1)
 - 152 van Seters AP & Moolenaar AJ. Mitotane increases the blood levels of hormone-binding proteins. *Acta Endocrinologica* 1991 **124** 526–533. (doi:10.1530/acta.0.1240526)
 - 153 Nader N, Raverot G, Emptoz-Bonneton A, Dechaud H, Bonnay M, Baudin E & Pugeat M. Mitotane has an estrogenic effect on sex hormone-binding globulin and corticosteroid-binding globulin in humans. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2165–2170. (doi:10.1210/jc.2005-2157)
 - 154 Alexandraki KI, Kaltsas GA, le Roux CW, Fassnacht M, Ajodha S, Christ-Crain M, Akker SA, Drake WM, Edwards R, Allolio B *et al.* Assessment of serum-free cortisol levels in patients with adrenocortical carcinoma treated with mitotane: a pilot study. *Clinical Endocrinology* 2010 **72** 305–311. (doi:10.1111/j.1365-2265.2009.03631.x)
 - 155 Boscaro M, Sonino N, Rampazzo A & Mantero F. Response of pituitary-adrenal axis to corticotrophin releasing hormone in patients with Cushing's disease before and after ketoconazole treatment. *Clinical Endocrinology* 1987 **27** 461–467. (doi:10.1111/j.1365-2265.1987.tb01174.x)
 - 156 McCance DR, Hadden DR, Kennedy L, Sheridan B & Atkinson AB. Clinical experience with ketoconazole as a therapy for patients with Cushing's syndrome. *Clinical Endocrinology* 1987 **27** 593–599. (doi:10.1111/j.1365-2265.1987.tb01189.x)
 - 157 Engelhardt D, Jacob K & Doerr HG. Different therapeutic efficacy of ketoconazole in patients with Cushing's syndrome. *Klinische Wochenschrift* 1989 **67** 241–247. (doi:10.1007/BF01717326)
 - 158 Tabarin A, Navarranne A, Guerin J, Corcuff JB, Parneix M & Roger P. Use of ketoconazole in the treatment of Cushing's-disease and ectopic ACTH syndrome. *Clinical Endocrinology* 1991 **34** 63–69. (doi:10.1111/j.1365-2265.1991.tb01737.x)
 - 159 Winquist EW, Laskey J, Crump M, Khamsi F & Shepherd FA. Ketoconazole in the management of paraneoplastic Cushing's syndrome secondary to ectopic adrenocorticotropin production. *Journal of Clinical Oncology* 1995 **13** 157–164.
 - 160 Berwaerts JJ, Verhelst JA, Verhaert GC, Verhaegen AA & Abs RE. Corticotropin-dependent Cushing's syndrome in older people: presentation of five cases and therapeutical use of ketoconazole. *Journal of the American Geriatrics Society* 1998 **46** 880–884.
 - 161 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 **77** 735–742. (doi:10.1111/j.1365-2265.2012.04424.x)
 - 162 Doi M, Sugiyama T, Izumiyama H, Yoshimoto T & Hirata Y. Clinical features and management of ectopic ACTH syndrome at a single institute in Japan. *Endocrine Journal* 2010 **57** 1061–1069. (doi:10.1507/endocrj.K10E-265)

Received 21 November 2014

Revised version received 8 January 2015

Accepted 29 January 2015