Steroidogenesis enzyme inhibitors in Cushing’s syndrome

Eleni Daniel¹ and John D C Newell-Price²

¹Department 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.

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 hypercortisolism 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 hypercortisolism 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
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
anologue, 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 manage-
ment 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 glucocorti-
coid suppression with KTZ both in vitro and in vivo, con-
fiming its inhibitory effect on adrenal steroidogenesis.

In the adrenal cortex, KTZ blocks multiple steps of
steroid biosynthesis through the inhibition of cytochrome
cp450 enzymes 17α-hydroxylase, 20,22-desmolase (22),
11β-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 hypo-
gonadism 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, hypo-
alkaemia, glycaemic control and hirsutism. Somewhat less
favourable data from France in 38 patients with CS
achieved normalisation 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,
average 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 recom-
ended 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 absorbence is enhanced when adminis-
tered 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 reac-
tions, male hypogonadism and gynaecomastia (32).
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 infections (17α-OH, 17α-hydroxylase; 3βHSD, 3β-hydroxysteroid dehydrogenase; 21-OH, 21-hydroxylase; 11β-OH, 11β-hydroxylase; 18-OH, 18-hydroxylase; *aldosterone synthase).

Figure 1
Steroidogenesis in the adrenal cortex denoting the specific pathways inhibited by ketoconazole (KTZ), metyrapone (MTR), mitotane, etomidate and newer steroidogenesis inhibitors

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).

Table 1 Steroidogenesis enzymes and their genes.

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

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

Reference: European Journal of Endocrinology
Steroidogenesis enzyme inhibitors in Cushing’s syndrome

E Daniel and J D C Newell-Price

R266
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\(\beta\)-hydroxylase, it also inhibits 18-hydroxylase (Fig. 1), and recent \textit{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
Table 3  Proposed dosage and monitoring regimens for the steroidogenesis inhibitors in the management of hypercortisolism.

<table>
<thead>
<tr>
<th>Steroidogenesis enzyme inhibitor</th>
<th>Dosage</th>
<th>Biochemical monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>Starting dose 400 mg can increase up to 1600 mg daily in two or three divided doses</td>
<td>1. UFC or cortisol day-curve at day 3, 8, 14, 21 and 35. Adjust dose if needed and recheck in 1 week</td>
</tr>
<tr>
<td>Metyrapone</td>
<td>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</td>
<td>2. Liver function tests weekly for the first month of treatment and then monthly</td>
</tr>
<tr>
<td>Etomidate</td>
<td>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)</td>
<td>1. UFC or cortisol day-curve at day 3, 8, 14, 21 and 35</td>
</tr>
<tr>
<td>Mitotane</td>
<td>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</td>
<td>2. Adjust dose according to biochemical response by 250–500 mg per dose and recheck biochemistry in 1 week</td>
</tr>
<tr>
<td></td>
<td>Low-dose regimen: start with 1 g daily and increase by 0.5 g every 72 h to a dose of 3 g daily</td>
<td>3. Adjust etomidate dose according to cortisol-lowering effect and level of sedation</td>
</tr>
<tr>
<td></td>
<td>Maximum dose is 12 g but high doses may not be tolerated</td>
<td>1. Check serum cortisol daily and after every dose change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Nurse in a High Dependency Unit and monitor for sedation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Adjust etomidate dose according to cortisol-lowering effect and level of sedation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Make adjustments of the dose by 1.5 g according to tolerance and plasma levels. If levels &gt;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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Use double-dose steroid replacement and avoid dexamethasone as the replacement glucocorticoid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Monitor TFTs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Monitor hypogonadism clinically</td>
</tr>
</tbody>
</table>

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 hypercortisolism 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.

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

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.
such as 11β-hydroxylase, 18-hydroxylase and 5α-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 fasciculare, 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 5α-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 11β-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 11β-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...
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 hypercortisolaeia. 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 antisteroidogenenic 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 11β-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 11β-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
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 11β-hydroxylase currently available. However, it causes an increase in ACTH that drives significant 11-deoxycortisosterone 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 11β-hydroxylase, 20,22-desmolase and 17α,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 hyperandrogenaemia 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
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 successful (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...
with one of the rapid-acting steroidogenesis inhibitors may be essential to provide early control of hypercortisolism 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 were hypercortisolism is 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 hypercortisolism 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 hypercortisolism, 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 hypercortisolism.

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 adenocortical 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 adenocortical 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 hypercortisolism 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 latter 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 11β-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 antihypertensives, 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


17 Feldman D. Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. Endocrine Reviews 2018 8 409–420. (doi:10.1209/edrv-7-4-409)


31 Van Der Meer JW, Keuning JJ, Scheijgrond HW, Heykants J, Van Cutsem J & Brugmans J. The influence of gastric acidity on the


50 Nelsson A & Woodard G. Severe adrenal cortical atrophy (cytotoxic) and hepatic damage produced in dogs by feeding 2,2-bis(parachlorophenyl)-1,1-dichlorothane (DDD or TDE). *Archives of Pathology* 1949 **48** 387–394.


132 Padmanabhan V, Keetch 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)


134 Amado JA, Pesquera C, Gonzalez EM, Otero M, Freijanes J & Alvarez A. Successful treatment with ketoconazole of Cushing’s syndrome in