Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5α-reductase, explaining the need for personalized glucocorticoid and androgen replacement.

MITOTANE THERAPY IN ADRENOCORTICAL CANCER INDUCES CYP3A4 AND INHIBITS 5A-REDUCTASE, EXPLAINING THE NEED FOR PERSONALIZED GLUCOCORTICOID AND ANDROGEN REPLACEMENT.

J Clin Endocrinol Metab. ***Vol. 98(1):161-71; 2013 Jan; doi: 10.1210/jc.2012-2851***

Mitotane Therapy in Adrenocortical Cancer Induces CYP3A4 and Inhibits 5_-Reductase, Explaining the Need for Personalized Glucocorticoid and Androgen Replacement

Vasileios Chortis,* Angela E. Taylor,* Petra Schneider, Jeremy W. Tomlinson, Beverly A. Hughes, Donna M. O'Neil, Rossella Libe´ , Bruno Allolio, Xavier Bertagna, Je´ roˆme Bertherat, Felix Beuschlein, Martin Fassnacht,

Niki Karavitaki, Massimo Mannelli, Franco Mantero, Giuseppe Opocher, Emilio Porfiri, Marcus Quinkler, Mark Sherlock, Massimo Terzolo, Peter Nightingale, Cedric H. L. Shackleton, Paul M. Stewart, Stefanie Hahner,

and Wiebke Arlt†

Context: Mitotane [1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane] is the first-line treatment for metastatic adrenocortical carcinoma (ACC) and is also regularly used in the adjuvant setting after presumed complete removal of the primary tumor. Mitotane is considered an adrenolytic substance, but there is limited information on distinct effects on steroidogenesis. However, adrenal insufficiency

and male hypogonadism are widely recognized side effects of mitotane treatment.

Objective: Our objective was to define the impact of mitotane treatment on in vivo steroidogenesis in patients with ACC.

Setting and Design: At seven European specialist referral centers for adrenal tumors, we analyzed 24-h urine samples (n_127) collected from patients with ACC before and during mitotane therapy in the adjuvant setting (n _ 23) or for metastatic ACC (n _ 104). Urinary steroid metabolite excretion was profiled by gas chromatography/mass spectrometry in comparison with healthy controls (n _ 88).

Results: We found a sharp increase in the excretion of 6_-hydroxycortisol over cortisol (P_0.001), indicative of a strong induction of the major drug-metabolizing enzyme cytochrome P450 3A4. The contribution of 6_-hydroxycortisol to total glucocorticoid metabolites increased from2%(median, interquartile range 1–4%) to 56% (39–71%) during mitotane treatment. Furthermore, we documented

strong inhibition of systemic 5_-reductase activity, indicated by a significant decrease in 5_-reduced steroids, including 5_-tetrahydrocortisol, 5_-tetrahydrocorticosterone, and androsterone (all P _ 0.001). The degree of inhibition was similar to that in patients with inactivating 5_-reductase type 2 mutations (n _ 23) and patients receiving finasteride (n _ 5), but cluster analysis of steroid data revealed a pattern of inhibition distinct from these two groups. Longitudinal data showed rapid onset and long-lasting duration of the observed effects.

Conclusions: Cytochrome P4503A4induction by mitotane results in rapid inactivation of more than 50% of administered hydrocortisone, explaining the need for doubling hydrocortisone replacement in mitotane-treated patients. Strong inhibition of 5_-reductase activity is in line with the clinical observation of relative inefficiency of testosterone replacement in mitotane-treated men, calling for replacement by 5_-reduced androgens.

Adrenocortical carcinoma (ACC) is a rare cancer, with an incidence of one to two cases per million per year and a poor prognosis, mostly due to a high risk of recurrence and limited therapeutic options (1). Mitotane [1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane (o,p_-DDD)], an analog of the insecticide dichlorodiphenyltrichloroethane (DDT), has been used in the treatment of ACC since 1959 (2). Mitotane alone or in combination with cytotoxic chemotherapy is now established as the first-line treatment for metastatic ACC (3– 8) and is also widely used as adjuvant therapy in patients with apparently complete surgical removal of the primary tumor, especially if considered at high risk of recurrence (9).

Despite the widespread use of mitotane in adrenal cancer, there is limited knowledge regarding the mechanisms underlying its antitumor activity, usually described as adrenolytic, i.e. a direct cytotoxic effect on the adrenal cortex (3, 10–12). There is also a paucity of information on distinct effects of mitotane on steroidogenesis, although it has been noted early on as an efficient treatment for Cushing's syndrome (13–16), and in patients with normal adrenal function, mitotane therapy invariably results in adrenal insufficiency. There is in vivo evidence of enhanced production of cortisolbinding globulin and SHBG in mitotane-treated patients (17, 18). Notably, glucocorticoid replacement has to be administered in higher doses than usual in the general context of adrenal insufficiency to prevent adrenal crisis (3, 19). Mitotane-induced hypogonadism is frequently observed in male patients (18), but testosterone

replacement often lacks clinical efficacy.

We have recently shown that urine steroid metabolomics, i.e. the combination of steroid profiling by gas chromatography (GC)/mass spectrometry (MS) and computational data analysis, is a highly promising diagnostic tool for the detection of adrenocortical malignancy (20). Here we investigated the effects of mitotane on in vivo steroid production employing urinary steroid metabolomics for the analysis of 24-h urine samples from patients with adrenal cancer receiving mitotane for adjuvant treatment or metastatic disease.

Subjects and Methods

Subjects

The 24-h urine samples from ACC patients were collected between 2006 and 2010 in seven specialist endocrine referral centers participating in the European Network for the Study of Adrenal Tumors (ENSAT; www.ensat.org), with approval of local ethical review boards and after obtaining written informed patient consent. We included 24-h urines from 100 patients (53 women, 47 men; median age 52, range 16–80 yr) with histologically confirmed ACC who provided a total of 127 samples including 46 paired samples. Samples were collected before (ADJ, n _ 12) and during (ADJ_M, n _ 11) adjuvant mitotane therapy or before (MET, n_57) and during (MET_M, n_47) mitotane treatment for metastatic ACC. Samples during mitotane treatment were collected 3–4 months after initiation of therapy, i.e. at a time when therapeutic-range plasma mitotane levels (14–20 mg/liter) (21) generally had been achieved. None of the patients on adjuvant therapy had documented recurrence during this initial treatment period, and there were no major changes in tumor burden as documented by imaging in the metastatic group

patients. Plasma mitotane levels were available for 50 of the 58 patients on mitotane, all of them measured by HPLC (Lysosafe, Paris, France). Exclusion criteria included pregnancy and exposure to drugs known to induce expression and activity of hepatic cytochrome P450 (CYP) enzymes or to alter steroid secretion in any way, with the exception of glucocorticoid replacement therapy, which was routinely commenced in all mitotane-treated patients.

For comparison, we employed 24-h urine samples of 88 healthy controls (62 females, 26 males, age range 18–60 yr). In addition, for the assessment of 5_-reductase activity, we also compared the results with 24-h urine samples from patients with inactivating mutations in SRD5A2 encoding 5_-reductase type 2 (n _ 23) and patients treated with the 5_-reductase type 2 inhibitor finasteride (n _ 5).

GC/MS urinary steroid metabolome analysis

Measurement of 24-h urinary steroid metabolite excretion was carried out by GC/MS as previously described (20). In summary, free and conjugated steroids were extracted from urine by solid-phase extraction. Steroid conjugates were enzymatically hydrolyzed, reextracted, and chemically derivatized to form methyloxime trimethyl silyl ethers. GC/MS analysis of the urine samples was carried out on an Agilent (Santa Clara, CA) 5973 instrument operating in selected-ion-monitoring (SIM) mode. This achieved sensitive and specific detection and quantification of 32 selected steroid metabolites chosen to include important representatives of steroid groups such as androgen metabolites, glucocorticoid metabolites, mineralocorticoid metabolites, and 3_-hydroxy-_ (5) steroid metabolites (for details of the steroid metabolite profile see Ref. 20).

After analysis of the entire profile, we calculated substrate metabolite to product metabolite ratios to assess the effects of mitotane on the in vivo net activity of distinct steroidogenic enzymes.

This included 5_-reductase indicated by the ratio of 5_-tetrahydrocortisol to tetrahydrocortisol (5_-THF/THF), ratio of androsterone to etiocholanolone (An/Et), and ratio of 5_-tetrahydrocorticosterone/ tetrahydrocorticosterone (5_-THB/THB).

As a measure of the activity of the major drug-metabolizing enzyme CYP3A4, we calculated the ratio of 6_-hydroxycortisol to cortisol (6_OHF/F) (22). Total steroid output was calculated as the sum of all quantified steroid metabolites with the exception of glucocorticoid metabolites because these also reflected exogenously administered glucocorticoid replacement. Total when used in this paper relates to the targeted compounds measured, which are dominant metabolites of hormonal steroids and their precursors. It does not include a multitude of minor metabolites.

Statistical analysis

Diagnostic ratios were presented as median and interquartile ranges [quartile 1 (Q1)–Q3]. In a first analysis, we considered all samples to be independent and employed nonparametric Kruskal-Wallis test and Dunn's post hoc test to detect significant differences of individual ratios among the treatment groups. To take the paired nature of a subset of samples into account, we provided a separate analysis of the data employing Wilcoxon signed rank test. These analyses were carried employing Sigma-Plot (Systat Software Inc., Chicago, IL).

Furthermore, we analyzed the influence of mitotane on 5_-reductase activity by performing multivariate analyses of the ratios reflecting 5_-reductase activity, An/Et, 5_-THF/THF, and 5_-THB/THB. We performed principal component analysis (PCA) and linear discriminant analysis (LDA) (23) to generate two-dimensional representations of the data. Prior to analysis, the values of the ratios were log-transformed and normalized to zero mean and unit variance. The generated scatter plots allowed identification of clusters of similar ratio profiles. These analyses were done using the software MATLAB (Mathwork Inc.,

Natick, MA).

Finally, we analyzed the association between plasma mitotane levels and the values of the steroid ratios indicative of 5_-reductase andCYP3A4activities by computing Spearman's rank correlation coefficient, thereby accounting for the lack of normal distribution of the data. This analysis was carried out employing MATLAB.

Results

Mitotane down-regulates overall steroidogenesis

First, we analyzed total steroid excretion to assess whether mitotane has an impact on the initial steps of steroidogenesis, namely CYP11A1 activity converting cholesterol to pregnenolone. For this analysis, we disregarded active glucocorticoid metabolites because the mitotane-treated patients invariably received hydrocortisone replacement therapy, which prevented a comprehensive assessment of endogenous glucocorticoid production. Comparing the remainder of total steroid excretion, we found that mitotane led to a significant down-regulation of overall steroidogenesis in metastatic ACC patients, as documented by a significant decrease in the sum of total androgen and mineralocorticoid metabolites (Fig. 1A and Table 1). This downregulation was significant for the larger group of metastatic ACC patients, decreasing both androgen and mineralocorticoid excretion to the level found in healthy controls, but failed to reach significance for the smaller adjuvant therapy group (Fig. 1A and Table 1). Of note, we found that the excretion of the 11-deoxycortisol metabolite tetrahydro-11-deoxycortisol did not

differ when comparing steroid excretion before and after the initiation of mitotane therapy (Fig. 1B and Table 1), indicating that mitotane had no effect on 11_-hydroxylase activity, which converts 11-deoxycortisol to cortisol.

Mitotane induces CYP3A4 activity and glucocorticoid inactivation

After the initiation of mitotane treatment, 6_OHF/F showed a significant increase in both patients with metastatic disease and patients receiving adjuvant therapy (Fig. 1, C and D, and Table 1 and Supplemental Table 1, published on The Endocrine Society's Journals Onlinewebsite at http://jcem.endojournals.org), which was due to large increases in 6_-OHF excretion, indicative of a highly enhanced rate of inactivation of cortisol to 6_OHF, a conversion predominantly catalyzed by CYP3A4 (24–26).

Before mitotane treatment, 6_OHF represented only 1.3% (ADJ; Q1–Q3 1.0–2.6%) and 1.8% (MET; Q1–Q3 0.8–4.2%), respectively, of total measured glucocorticoid metabolite excretion. By contrast, during mitotane treatment, 63.8% (ADJ_M;Q1–Q348.9–70.2%) and 52.5% (MET_M; Q1–Q3 38.8–69.1%), respectively, of measured glucocorticoids were excreted as 6_OHF (Fig. 1E), suggesting rapid inactivation of exogenously administered hydrocortisone in the mitotane-treated patients.

To exclude a significant contribution of the concurrent hydrocortisone replacement on the induction of CYP3A4 observed in the mitotane-treated patients, we also studied the percentage of 6_OHF as part of total glucocorticoid excretion in patients with adrenal insufficiency, specifically 30 patients on a regular-dose hydrocortisone replacement (10–30 mg/d) and 10 patients who received

400 mg hydrocortisone during the 24-h period of urine collection. Results revealed that 6_OHF on regular hydrocortisone dose did not differ from healthy controls, whereas high-dose hydrocortisone slightly increased the median percentage of 6_OHF to just under 5% (Fig. 1E), confirming an only very minor contribution of hydrocortisone to the observed effect.

GC/MS analysis of urine also showed a major increase in the excretion of normally minor metabolites formed throughCYP3A4activity but not selected for quantitation in this study, notably 6_- and 1_-hydroxylated metabolites of tetrahydrocortisone and the cortolones.Wedid not find an effect of mitotane on the steroid ratios reflective of 11_-hydroxysteroid dehydrogenase type 1 or 2 activity.

Mitotane inhibits 5_-reductase activity and androgen activation

Introduction of mitotane therapy resulted in a highly significant decrease of several steroid metabolite ratios reflective of systemic 5_-reductase activity (Fig. 2 and Table 1 and Supplemental Table 1). The degree of inhibition of 5_-reductase activity appeared to be similar to that observed in patients receiving treatment with the established 5_-reductase type 2 inhibitor finasteride (n_5) and patients with inactivating 5_-reductase type 2 (SRD5A2) mutations (n_25) (Fig. 2 and Table 2). However, of note, the 5_-THB/THB ratio was more significantly inhibited by mitotane than observed in finasteride-treated or SRD5A2 mutant patients (Fig. 2 and Table 2).

To examine the pattern of inhibition of 5_-reductase in further detail, we carried out cluster analysis employing both LDA and PCA; for this analysis, we considered all

three ratios reflective of 5_-reductase activity and selected the patients receiving mitotane in the adjuvant setting to exclude any influence of tumor-related steroid production. Visualization of the data convincingly demonstrated strongly overlapping clustering of finasteride-treated patients and SRD5A2 mutant patients, who both have selective loss of 5_-reductase type 2 activity (Fig. 3A), indicative of similar ratio profiles in these two groups. By contrast, patients receiving mitotane are clearly separate in a second cluster (Fig. 3A). These findings were confirmed by an independent cluster analysis employing LDA (Fig. 3B).

Longitudinal studies

We analyzed the longitudinal course of the steroid ratios indicative of CYP3A4 activity (Fig. 4A) and 5_-reductase activity (Fig. 4, B and C) in five patients receiving adjuvant mitotane therapy for adrenocortical cancer. Results demonstrate a rapid onset of the effects of mitotane on the enzymatic activities of CYP3A4 and 5_-reductase, with the full extent of the effect already documented shortly after initiation of mitotane treatment (Fig. 4, A–C).

We had the opportunity to document the diagnostic steroid ratios in one patient throughout 2 yr of adjuvant mitotane treatment followed by 2 yr of posttreatment observation (Fig. 4D). Plasma mitotane levels oscillated within the suggested therapeutic range (14–20 mg/liter) throughout the treatment period and only became undetectable 1 yr after the last administration of mitotane.

Concurrently, the steroid ratios indicative ofCYP3A4and 5_-reductase activity started to recover but had not returned to pretreatment levels even 2 yr after the end of treatment (Fig. 4D), suggestive of long-lasting effects.

Plasma mitotane levels and observed effects on steroidogenesis

We analyzed the correlation between circulating plasma mitotane levels and the severity of CYP3A4 induction and 5_-reductase inhibition, respectively (Supplemental Fig. 1). This revealed a significant correlation between plasma mitotane levels and the induction of CYP3A4 [r _ 0.328, 95% confidence interval (CI) _ 0.055–0.556, P _ 0.02] but not with the steroid ratios indicative of 5_-reductase activity (r__0.053,95%CI_ _0.327–0.229, P _ 0.71 for 5_THF/THF; r _ 0.106, 95% CI__0.178–0.373, P _ 0.46 for An/Et) (Supplemental Fig. 1). Significant effects on the enzymatic activities were already observed at very low plasma mitotane levels and clearly below the suggested therapeutic range of 14–20 mg/liter (21) (Supplemental Fig. 1). These findings are in line with the above-described observation that urine metabolite excretion showed the full effects as early as 1–2 months after initiation of mitotane treatment (Fig. 4, A–C), when in most instances, plasma mitotane levels would not have reached the therapeutic range.

Discussion

This study documented comprehensive in vivo evidence for a strong inhibition of 5_-reductase activities and a significant induction of hepatic CYP3A4/5 activities in mitotane-treated patients, with an obvious and important impact on the requirements for glucocorticoid and androgen replacement during mitotane therapy.

Evidence for alterations of cortisol metabolism and a link to hepatic enzyme activity was documented shortly

after the introduction of mitotane for the treatment of adrenal cancer in 1959 (2). In 1964, two groups reported on altered cortisol metabolism resulting in increased urinary excretion of 6_OHF in guinea pig (27) and humans (28), respectively. Following up on these reports, two groups documented increased metabolism of pentobarbital (29) and hexobarbital and cortisol (30) by mitotane, postulating the induction of microsomal drug-metabolizing liver enzymes as the underlying cause. Work in the late 1980s demonstrated that the major drug-metabolizing enzyme CYP3A4 and to a lesser degree also CYP3A5 were the enzymes responsible for 6_-hydroxylation in liver and kidney (24 –26). Subsequently, the urinary 6_OHF/F has been widely implemented as a relative measure of CYP3A4/5 in vivo activity and several studies demonstrated a 4- to 7-fold increase in 6_OHF excretion in patients treated with rifampicin (25) or anticonvulsants (31). This study analyzing 24-h urine samples from 127 patients collected before and during mitotane treatment demonstrated a 10- to 15-fold increase in 6_OHF/F excretion, establishing mitotane as one of the strongest inducers of CYP3A4 activity. This convincingly corroborates a recent report on accelerated midazolam metabolism in four mitotane-treated patients suggestive of induction of CYP3A4 (and CYP3A5) activity (32).

Early reports on hepatic enzyme induction by mitotane and also the invariable finding of highly increased cortisolbinding globulin levels during mitotane treatment (17) suggested an increased dose requirement for glucocorticoid replacement in mitotane-induced adrenal insufficiency.

However, this has been widely recognized only in recent years, after a number of reports on adrenal crisis during mitotane treatment covered only with standard glucocorticoid replacement doses (19, 33). Consequently, the perceived rate of reported gastrointestinal toxicity during mitotane treatment has declined over recent years, because many of these signs and symptoms may have been reflective of incipient adrenal crisis. However, currently, there is no uniformly agreed dose regimen for glucocorticoid replacement during mitotane therapy and reported doses have varied widely (17, 18), with lower doses often reported as associated with a high incidence of vomiting and severe fatigue (18, 34). Our findings provide for the first time a reliable quantification of 6_-hydroxylation by mitotane, documenting the rapid excretion of 55–65% of glucocorticoids in the form of 6_OHF.

Of note, CYP3A4/5 are responsible for not only the conversion of cortisol to 6_OHF but also for the 6_-hydroxylation of testosterone. Hypogonadism in mitotane-treated men initially manifests with a decreased free androgen index due to significantly increased SHBG levels that cannot be compensated for by up-regulated testosterone production as documented by increased total testosterone and LH levels (35). With time, gonadal testosterone production exhausts itself and circulating testosterone levels drop, accompanied by clinical manifestations of low testosterone including erectile dysfunction. However, testosterone replacement is often clinically ineffective and is complicated by an increased rate of gynecomastia (18).

This study has yielded comprehensive evidence for a strong inhibition of 5_-reductase activities by mitotane. Importantly,

the strong inhibition of 5_-reductase has significant consequences for androgen bioactivity, because the conversion of testosterone to the most potent androgen, 5_-dihydrotestosterone, will be greatly reduced. Consequently, thismayresult in enhanced conversion of testosterone to 17_-estradiol by widespread CYP19A1 (P450 aromatase) activity, which could explain the high incidence of gynecomastia in mitotane-treated patients. The lack of conversion of testosterone to 5_-dihydrotestosterone also represents a logical explanation for the frequent clinical observation of relative inefficiency of testosterone replacement with regard to erectile dysfunction. Our computational analysis of the ratios of 5_- to 5_-reduced steroids revealed a distinct pattern of global 5_-reductase inhibition by mitotane compared with patients treated with a selective 5_-reductase type 2 inhibitor or patients with inactivating5_-reductase type2mutations.These results could indicate preferential inhibition of 5_-reductase type 1 by mitotane. 5_-Reductase inhibition could also have beneficial consequences in the context of androgen-producing ACC, where it would be likely to help ameliorate the clinical manifestations of androgen excess.

A number of early studies addressed the impact of mitotane on adrenal steroidogenesis, reporting inhibitory effects of mitotane on 11_-hydroxylase, 3_-hydroxysteroid dehydrogenase, and 18-hydroxylase activities (36–39). However, in vivo studies by labeled isotope infusion were very limited in numbers, whereas in vitro studies were somewhat limited in their methodological approach. In our study, we found no evidence for distinct enzyme inhibition other than the above described strong inhibition of 5_-reductase activities and the induction of CYP3A4/5.

Specifically, there was no change in 11-deoxycortisol metabolite excretion, rendering a significant change in 11_-hydroxylase (CYP11B1) enzymatic activity highly unlikely. However, we observed a down-regulation of overall steroidogenesis as quantified by the sum of total androgen and mineralocorticoid excretion; glucocorticoid metabolites were excluded for that analysis as altered due to the mandatory exogenous hydrocortisone replacement in mitotane-treated patients. These findings could indicate an inhibition of CYP11A1, i.e. P450 side-chain cleavage enzyme, as previously described in vitro (40), which would result in decreased conversion of cholesterol to pregnenolone and thus a decreased substrate entry flow into the steroidogenic pathways. This could contribute to the hypercholesterolemia that is a widely documented side effect of mitotane treatment and that has been previously suggested to be due to increased cholesterol synthesis as a consequence of mitotane-induced up-regulation of 3-hydroxy-3-methyl-glutarylcoenzyme A-reductase activity (41, 42).

Our study provides a quantifiable measure for the strong induction of CYP3A4/5 activities by mitotane, which has the clinically most relevant consequences, including potential drug interactions in mitotane-treated patients, nicely summarized in a recent review (43). This has an impact not only on drugs needed for the treatment of mitotane-related side effects but, importantly, also on antitumor drugs including tyrosine kinase and mammalian target of rapamycin inhibitors and also chemotherapeutic agents included in the current first-line treatment for metastatic adrenal cancer (44).

Our findings of strong and long-lasting inhibition of 5_-reductase and induction ofCYP3A4/5refine our understanding of the requirements for steroid replacement therapy in mitotane-treated patients. Our observation of a very rapid induction of CYP3A4 by mitotane suggests that lower glucocorticoid replacement doses, such as 25 mg cortisone acetate (18) equivalent to 15 mg hydrocortisone (45), may soon become inadequate and contribute to the gastrointestinal toxicity observed during the first months of mitotane treatment. Thus, we suggest that glucocorticoid replacement in mitotanetreated patients should be initiated and maintained with at least double the dose normally used in primary adrenal insufficiency, i.e. 40–50 mg hydrocortisone (equal to 75 mg cortisone acetate) rather than 20–25 mg hydrocortisone (equal to 37.5 mg cortisone acetate) per day. Dexamethasone should be avoided because it exerts a strong CYP3A4/5-inducing effect (46) that is likely to result in even more rapid inactivation. Whether urinary 6_OHF excretion can be used as a guide for dose adjustment will have to be examined by prospective studies. At present, the appropriateness

of glucocorticoid replacement during mitotane therapy largely relies on clinical assessment and plasma ACTH measurements after the morning hydrocortisone dose, with increased levels suggestive of glucocorticoid underreplacement. Importantly, we should consider the use of 5_-reduced androgens, including synthetic androgens, for androgen replacement in mitotane-induced male hypogonadism, which may prove more effective and less prone to unwanted side effects than testosterone replacement therapy. Pregnancy needs to be added to the list of contraindications for mitotane therapy, and patients should have safe contraception in place because the strong inhibition of 5_-reductase activity would have a major impact on sexual differentiation, with a high likelihood of disordered sex development in the male fetus.

Acknowledgments

We are grateful to Amar Agha and Lucy Ann Behan, Beaumont Hospital, Dublin, Ireland, for access to 24-h urine samples from patients receiving regular dose hydrocortisone replacement for adrenal insufficiency.

Address all correspondence and requests for reprints to: Prof Wiebke ArltMDDScFRCPFMedSci, Centre for Endocrinology, Diabetes and Metabolism, School of Clinical & Experimental Medicine, University of Birmingham, Birmingham, B15 2TT, United Kingdom, Tel. _44 121 415 8716, Fax _44 121 415 8712, E-mail: w.arlt@bham.ac.uk.

This work was supported by the Medical Research Council UK (Strategic Grant G0801473 to W.A. and P.M.S.), the European Union under the Seventh Framework Program (FP7/2007-2013, Grant Agreement 259735, ENSAT-CANCER), the National Institute of Health ResearchUK(NIHRAcademic Clinical Fellowship, to V.C.), and the Claire Khan Adrenal Trust Fund.

Disclosure Summary: The authors have nothing to disclose.

References

1. Fassnacht M, Libe´ R, Kroiss M, Allolio B 2011 Adrenocortical carcinoma: a clinician's update. Nat Rev Endocrinol 7:323–335

2. Bergenstal DM, Lipsett M, Moy RH, Hertz R 1959 Regression of adrenal cancer and suppression of adrenal function in man by o,p_-DDD. Trans Am Physicians 72:341–350

3. Hahner S, Fassnacht M 2005 Mitotane for adrenocortical carcinoma treatment. Curr Opin Investig Drugs 6:386–394

4. Hutter JrAM,KayhoeDE1966 Adrenal cortical carcinoma. Results of treatment with o,p_DDD in 138 patients. Am J Med 41:581–592

5. Lubitz JA, Freeman L, Okun R 1973 Mitotane use in inoperable adrenal cortical carcinoma. JAMA 223:1109–1112

6. Jarabak J, Rice K 1981 Metastatic adrenal cortical carcinoma. Prolonged regression with mitotane therapy. JAMA 246:1706–1707

7. Luton JP, Cerdas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P 1990 Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. N Engl J Med 322:1195–1201

8. Wooten MD, King DK 1993 Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. Cancer 72:3145–3155

9. Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi G, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L, BerrutiA2007 Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med 356:2372–2380

10. Vilar O, Tullner WW 1959 Effects of o,p_DDD on histology and 17-hydroxycorticosteroid output of the dog adrenal cortex. Endocrinology 65:80–86

11. Kaminsky N, Luse S, Hartroft P 1962 Ultrastructure of adrenal cortex of the dog during treatment with DDD. J Natl Cancer Inst 29:127–159

12. Jensen BL, Caldwell MW, French LG, Briggs DG 1987 Toxicity, ultrastructural effects, and metabolic studies with 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane(o,p_-DDD) and its methyl analog in the guinea pig and rat. Toxicol Appl Pharmacol 87:1–9

13. Southren AL, Tochimoto S, Strom L, Ratuschni A, Ross H, Gordon G 1966 Remission in Cushing's syndrome with o,p_-DDD. J Clin Endocrinol Metab 26:268–278

14. Temple Jr TE, Jones Jr DJ, Liddle GW, Dexter RN 1969 Treatment of Cushing's disease. Correction of hypercortisolism by o,p_DDD without induction of aldosterone deficiency. N Engl J Med 281: 801–805

15. Luton JP,MahoudeauJA, Bouchard P, Thieblot P, Hautecouverture M, Simon D, Laudat MH, Touitou Y, Bricaire H 1979 Treatment of Cushing's disease by o,p_DDD. Survey of 62 cases.NEngl J Med 300:459–464

16. Donadille B, Groussin L, Waintrop C, Abbas H, Tenenbaum F, Dugue´ MA, Coste J, Bertagna X, Bertherat J 2010 Management of Cushing's syndrome due to ectopic adrenocorticotropin secretion with 1,ortho-1, para'-dichloro-diphenyl-dichloro-ethane: findings in 23 patients from a single center. J Clin Endocrinol Metab 95: 537–544

17. Nader N, Raverot G, Emptoz-Bonneton A, De´chaud H, Bonnay M, Baudin E, PugeatM2006 Mitotane has an estrogenic effect on sex hormone-binding globulin and corticosteroid-binding globulin in humans. J Clin Endocrinol Metab 91:2165–2170

18. Daffara F, De Francia S, Reimondo G, Zaggia B, Aroasio E, Porpiglia F, Volante M, Termine A, Di Carlo F, Dogliotti L, Angeli A, Berruti A, TerzoloM2008 Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. Endocr Relat Cancer 15:1043–1053

19. Robinson BG, Hales IB, Henniker AJ, Ho K, Luttrell BM, Smee IR, StielJN1987 The effect of o,p_-DDDon adrenal steroid replacement therapy requirements. Clin Endocrinol (Oxf) 27:437–444

20. Arlt W, Biehl M, Taylor AE, Hahner S, Libe´ R, Hughes BA, Schneider

P, Smith DJ, Stiekema H, Krone N, Porfiri E, Opocher G, Bertherat J, Mantero F, Allolio B, Terzolo M, Nightingale P, Shackleton CH, Bertagna X, Fassnacht M, StewartPM2011 Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors. J Clin Endocrinol Metab 96:3775–3784

21. Hermsen IG, Fassnacht M, Terzolo M, Houterman S, den Hartigh J, Leboulleux S, Daffara F, Berruti A, Chadarevian R, Schlumberger M, Allolio B, Haak HR, Baudin E 2011 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. J Clin Endocrinol Metab 96:1844–1851

22. Galteau MM, Shamsa F 2003 Urinary 6_-hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. Eur J Clin Pharmacol 59:713–733

23. Duda RO, Hart PE, Stork DG 2000 Pattern classification. 2nd ed. New York: Wiley-Interscience

24. Waxman DJ, Attisano C, Guengerich FP, Lapenson DP 1988 Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6_-hydroxylase cytochrome P-450 enzyme. Arch Biochem Biophys 263:424–436

25. Ged C, Rouillon JM, Pichard L, Combalbert J, Bressot N, Bories P, Michel H, Beaune P, Maurel P 1989 The increase in urinary excretion of 6_-hydroxycortisol as a marker of human hepatic cytochrome P450IIIA induction. Br J Clin Pharmacol 28:373–387

26. Wrighton SA, Brian WR, Sari MA, Iwasaki M, Guengerich FP, Raucy JL, Molowa DT, Vandenbranden M 1990 Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). Mol Pharmacol 38:207–213

27. Kupfer D, Balazs T, Buyske DA 1964 Stimulation by o,p_-DDD of cortisol metabolism in the guinea pig. Life Sci 3:959–964

28. Bledsoe T, Island DP, Ney RL, Liddle GW 1964 An effect of o,p_-DDD on the extra-adrenal metabolism of cortisol in man. J Clin Endocrinol Metab 24:1303–1311

29. Straw JA, Waters IW, FreglyMJ1965 Effect of o,p_-DDDon hepatic

metabolism of phenobarbital in rats. Proc Soc Exp Biol Med 118:
391–394
30. Kupfer D, Peets L 1966 The effect of o,p_DDD on cortisol and
hexobarbital metabolism. Biochem Pharmacol 15:573–581
31. Saenger P, Forster E, Kream J 1981 6_-hydroxycortisol: a nonin-

vasive indicator of enzyme induction. J Clin Endocrinol Metab 52:
381–384
32. van Erp NP, Guchelaar HJ, Ploeger BA, Romijn JA, Hartigh J, GelderblomH2011
Mitotane has a strong and a durable inducing effect
on CYP3A4 activity. Eur J Endocrinol 164:621–626
33. Hague RV, May W, Cullen DR 1989 Hepatic microsomal enzyme
induction and adrenal crisis due to o,p_DDD therapy for metastatic
adrenocortical carcinoma. Clin Endocrinol (Oxf) 31:51–57
34. Hogan TF, Citrin DL, JohnsonBM,Nakamura S, Davis TE, Borden
EC 1978 o,p_-DDD (mitotane) therapy of adrenal cortical carcinoma:
observations on drug dosage, toxicity, and steroid replacement.
Cancer 42:2177–2181
35. Sparagana M 1987 Primary hypogonadism associated with o,p-
_DDD (mitotane) therapy. J Toxicol Clin Toxicol 25:463–472
36. Bradlow HL, Fukushima DK, Zumoff B, Hellman L, Gallagher TF
1963 A peripheral action of o,p_-DDD on steroid biotransformation.
J Clin Endocrinol Metab 23:918–922
37. Brown RD, Nicholson WE, Chick WT, Strott CA 1973 Effect of
o,p_DDD on human adrenal steroid 11_-hydroxylation activity.
J Clin Endocrinol Metab 36:730–733
38. Touitou Y, Bogdan A, Auzeby A, Dommergues JP 1979 Glucocorticoid
and mineralocorticoid pathways in two adrenocortical carcinomas:
comparison of the effects of o,p_-dichlorodiphenyldichloroethane,
aminoglutethimide and 2-p-aminophenyl-2-phenylethylamine in
vitro. J Endocrinol 82:87–94
39. Ojima M, Saitoh M, Itoh N, Kusano Y, Fukuchi S, Naganuma H
1985 [The effects of o,p_-DDD on adrenal steroidogenesis and hepatic
steroid metabolism]. Nihon Naibunpi Gakkai Zasshi 61:168–
178 (Japanese)

40. Kurokohchi K, Nishioka M, Ichikawa Y 1992 Inhibition mechanism of reconstituted cytochrome P-450scc-linked monooxygenase system by antimycotic reagents and other inhibitors. J Steroid Biochem Mol Biol 42:287–292

41. Stacpoole PW, Varnado CE, Island DP 1982 Stimulation of rat liver 3-hydroxy-3-methylglutaryl-coenzymeAreductase activity by o,p_-DDD. Biochem Pharmacol 31:857–860

42. Maher VM, Trainer PJ, Scoppola A, Anderson JV, Thompson GR, Besser GM 1992 Possible mechanism and treatment of o,p_DDDinduced hypercholesterolaemia. Q J Med 84:671–679

43. Kroiss M, Quinkler M, LutzWK,Allolio B, FassnachtM2011 Drug interactions with mitotane by induction of CYP3A4 metabolism in the clinical management of adrenocortical carcinoma. Clin Endocrinol (Oxf) 75:585–591

44. Fassnacht M, Terzolo M, Allolio B, Baudin E, Haak H, Berruti A, Welin S, Schade-Brittinger C, Lacroix A, Jarzab B, Sorbye H, Torpy DJ, Stepan V, Schteingart DE, Arlt W, Kroiss M, Leboulleux S, Sperone P, Sundin A, Hermsen I, Hahner S, Willenberg HS, Tabarin A, Quinkler M, de la Fouchardie`re C, et al. 2012 Combination chemotherapy in advanced adrenocortical carcinoma. N Engl J Med 366:2189–2197

45. Allolio B, Kaulen D, Deuss U, Hipp FX, WinkelmannW1985 Comparison between hydrocortisone and cortisone acetate as replacement therapy in adrenocortical insufficiency. Aktuelle Endokrinologie Stoffwechsel 6:35–39

46. Villikka K, Kivisto¨ KT, Neuvonen PJ 1998 The effect of dexamethasone on the pharmacokinetics of triazolam. Pharmacol Toxicol 83:135–138