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PRACTICAL TREATMENT USING MITOTANE FOR ADRENOCORTICAL CARCINOMA.

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ABSTRACT

Purpose of review

Description of novel findings about the mechanism of action of mitotane and its activity as an adjunctive post-operative measure, or for treatment of advanced adrenocortical carcinoma (ACC).

Recent findings

Several in vitro studies have shown that mitotane suppresses gene transcription of different enzymatic steps of the steroidogenetic pathway. Moreover, mitotane induces CYP3A4 expression thus accelerating the metabolic clearance of a variety of drugs including steroids. Retrospective studies provided evidence that adjunctive mitotane can prolong recurrence-free survival of treated patients. The concept of a therapeutic window of mitotane plasma concentrations was confirmed also for adjunctive treatment but the relationship between mitotane concentration and given dose is loose. Genetic variability of the P450-dependent enzymes metabolizing mitotane may explain individual differences.

Summary

Mitotane concentration of 14-20 mg/l should be reached and maintained during treatment also in an adjunctive setting. In advanced ACC, a high-dose starting regimen should be employed when mitotane is used as monotherapy. The combination of mitotane with other drugs should consider the possibility of pharmacologic interactions due to mitotane-induced activation of drug metabolism. This concept applies also to steroid replacement in mitotane-treated patients, who need higher doses to adjust for increased steroid metabolism.

Key words

Adjunctive treatment, adrenocortical carcinoma, mitotane, overall survival, recurrence-free survival.

INTRODUCTION

A limited range of therapeutic options is available for adrenocortical carcinoma (ACC). Both the rarity and aggressiveness of ACC concur to hamper progress in the development of treatment beyond surgery. Despite recent advancements, knowledge of the molecular pathways underlying ACC development and progression remains limited and up to now no effective target therapy entered use (1).

In this grim scenario, mitotane remains a cornerstone in the management of patients with ACC. More than 50 years have passed since mitotane was applied in clinical practice; however, we have still many uncertainties on how to use this old drug and what we may expect in terms of activity (2). Mitotane is currently used in post-operative adjuvant setting and in advanced disease. However, no results from randomized prospective trials on mitotane are available to guide management.

The objective of this work is to provide a concise review of recent advances in the use of mitotane. The most interesting articles published over the past 24 months dealt with the mechanism of action of mitotane and its practical use in the management of patients with ACC, and these topics will be addressed in the present review.

MECHANISM OF ACTION OF MITOTANE

Despite the widespread knowledge that mitotane has a profound effect on steroidogenesis (1-3), the specific mechanisms underlying its inhibitory effect are not fully understood. Mitotane action on adrenal steroidogenesis has been associated with the inhibition of a number of mitochondrial cytochrome P450-dependent enzymes: cholesterol side chain cleavage (CYP11A1), 11β-hydroxylase (CYP11B1), and 18β-hydroxylase (CYP11B2) (4, 5), as well as P450-independent enzymes, such as 3β-hydroxysteroid-dehydrogenase (6).

Fresh data on this topic come from the study of Lin et al. (7*), who explored the effect of non-cytotoxic concentrations of mitotane on cortisol production by NCI-H295 cells and found that mitotane interferes with gene transcription of a number of steroidogenic enzymes. StAR and CYP11A1, that are involved in the rate-limiting step of steroidogenesis, are most sensitive to mitotane (Figure 1). The effect on CYP11B1 was more stimulatory than inhibitory, contradicting early reports of a strong suppression of CYP11B1 activity (8).

The relationship between mitotane and steroidogenesis was also recently evaluated in other recent papers. Van Koetsveld et al (9) investigated the effect of mitotane and interferon-β in primary cultures of ACC and found that both drugs strongly inhibited mRNA expression of StAR, CYP11A1, CYP17A1, and CYP11B1. Combination of mitotane and interferon-β induced an additive inhibitory effect on cellular DNA number and cortisol secretion, suggesting that treatment with interferon-β may increase sensitivity of ACC cells to mitotane. Lehmann et al. (10) studied the effect of a 24-hour mitotane treatment on NCI-H295R cell viability and expression of genes involved in adrenal steroidosynthesis has been analyzed. It was found that mitotane markedly inhibited expression of genes coding for enzymes involved in generation of cortisol and dehydroepiandrosterone sulfate (CYP11A1 and CYP17A1). Moreover, mitotane reduced viability of NCI-H295R cells inducing cell apoptosis triggered by increased caspase 3 and caspase 7 activities. The mitotane-induced repression of genes of the steroidogenetic pathway has been confirmed by another study in the same cell line (11).

Chortis et al. (12**) studied the steroid inhibitory effect of mitotane in vivo, using a novel steroidobolomic approach to analyze 24-h urine samples from ACC patients receiving mitotane for adjuvant treatment or metastatic disease. It was found that mitotane down-regulated the initial steps of steroidogenesis but did not influence 11β-hydroxylase activity. As previously discussed, in vitro data are controversial about the mitotane effect on this enzymatic step. Moreover, mitotane was found to be a strong inducer of CYP3A4 activity leading to glucocorticoid inactivation and consequent sharp rise in 6β-hydroxycortisol urinary excretion. It was calculated that mitotane is

able to inactivate 50% of administered hydrocortisone and this explains why patients on mitotane have an increased dose requirement for steroid replacement. Finally, mitotane proved to be a strong inhibitor of 5α-reductase activity and this effect prompts to use 5α-dihydrotestosterone as androgen substitution in mitotane-treated men. An important mitotane-induced derangement of cortisol and testosterone metabolism has been also shown in a similar study (13).

To evaluate which are the intracellular targets of mitotane, Poli et al. (14*) performed electron microscopy on human ACC H295R cells and SW13 cell lines. Increasing concentrations of mitotane caused marked alterations in the morphology of mitochondria in a dose- and time-dependent manner. Mitochondria were finally disrupted leading to a drastic reduction of cell oxygen consumption. Mitotane was converted by the mitotane-sensitive H295R cells in its active metabolites and exerted cytostatic and cytotoxic effects at doses corresponding to the therapeutic window (30–50 μM). This study showed that mitotane effects seem to be mainly mediated by the mitochondria damage that activates an apoptotic process involving caspase 3 and caspase 7.

Further data showing that mitotane affects mitochondrial function have been reported by Hescot et al. (15*). In H295R and SW13 cell lines, mitotane inhibited cell proliferation in a dose- and a time-dependent manner and suppressed cortisol and 17-hydroxyprogesterone through inhibition of a number of genes involved in steroidogenesis (StAR, CYP11A1, HSD3B2, CYP11B1, and CYP11B2). Mitotane hampered the mitochondrial respiratory chain function complex IV (cytochrome coxidase) and this was accompanied by enhanced mitochondrial mass, as a compensatory mechanism in response to the respiratory chain defect. Furthermore, mitotane induced morphologic fragmentation of the mitochondrial membranes that are required for respiratory chain activity and presumably steroidogenesis.

MITOTANE FOR POST-OPERATIVE TREATMENT

The use of mitotane as an adjunctive post-operative treatment has attracted increasing attention following publication of a paper of ours showing that patients treated with adjuvant mitotane had a significantly longer recurrence-free survival (RFS) and overall survival (OS), compared with two independent groups of patients left untreated following surgery (16). This study raised also fierce criticisms due to its retrospective nature and inherent methodological limits (17).

A recent retrospective analysis of the management of patients with ACC at the University of Michigan, a tertiary referral center for ACC patients in the US, adds interesting data to this controversy (18**). Of 389 patients gathered form 1979 to 2013, 105 patients were treated post-operatively with mitotane and their outcome was compared with that of 159 patients receiving no adjunctive treatment. Despite that the adjuvant group had a worse risk profile than the control group, mitotane treatment was associated with a significantly improved RFS (HR=0.7, $P<0.05$). The beneficial effect of mitotane was confirmed in multivariable analysis. In the 42 patients receiving a combined adjunctive treatment consisting of post-operative radiotherapy of the local bed and mitotane, there was a positive interaction between the two treatments (HR=0.4, $P<0.05$) suggesting an additional benefit on RFS. However, both therapies failed to prolong significantly OS. The lack of effect on OS may be due to the relatively short follow-up duration (25.6 months in the overall series). Despite the usual limits of being a retrospective analysis, this paper has the merit of including a large cohort of well-characterized patients from a single center. Lacking data from controlled prospective trials, the results of this study add further evidence in favor of the use of mitotane in an adjuvant setting. The authors conclude that mitotane and radiotherapy may have a synergistic effect in reducing the risk of recurrence. However, radiotherapy was found to be ineffective in another retrospective analysis from US (19) and its role in the post-operative management of ACC patients remains even more controversial than mitotane.

Due to the referral pattern of the University of Michigan, which recruits most patients at the time of ACC recurrence, it is likely that the prognosis of ACC depicted in the study appears worse than it is. A study conducted in Germany has already observed a difference in the outcome of stage II ACC between patients referred to expert centers at diagnosis or after tumor recurrence. Patients who received early specialized care have a better prognosis and, interestingly, the use of adjuvant mitotane contributed to the difference (20).

Another recent retrospective paper provides additional albeit indirect evidence of the value of mitotane as an adjunctive post-operative treatment. This study correlated disease outcome with mitotane levels recorded in 122 patients with ACC who were radically operated on between 1995

and 2009 and were treated adjuvantly with mitotane at six European centers (21**). A monitored mitotane treatment targeting concentrations of 14–20 mg/l was done in all patients, but in only 63 of them (52%) the desired concentrations were reached and maintained during a median follow-up of 36 months. The patients with mitotane concentration at target showed a prolonged RFS compared with the remainders (HR of recurrence, 0.497; 95% CI, 0.292–0.844; P=0.01) while the increase in OS was of borderline statistical significance (HR of death, 0.511; 95% CI, 0.253–1.029; P=0.06) (Figure 2). The rather limited duration of follow-up and the low number of events may explain why OS was not significantly changed. Mitotane concentration of 14 mg/l, or higher, was a predictor of recurrence-free survival in multivariable analysis and this finding supports the concept of a therapeutic interval of mitotane concentratons, that was originally developed in the setting of advanced disease. The study validated the strategy of targeting a cutoff value of 14 mg/l when giving mitotane for adjunctive purpose (22), that was previously recommended on an expert opinion basis (1, 23, 24). However, the study demonstrated also that maintaining mitotane concentrations at target for a long time is a difficult task requiring firm commitment by either patients and physicians.

The patients included in this study were treated with different dosing regimens of mitotane, according to the policies at each center. However, there was no difference between low-dose and high-dose regimens in the probability of reaching the target concentrations after three months of treatment, suggesting that individual factors may be as important as pharmacologic ones (21). Treatment-related toxicity was overall acceptable and manageable with temporary treatment discontinuation, or dose reduction. Although a retrospective analysis may underestimates adverse events, it is likely that the monitoring of mitotane concentrations contributed to limit severe unwanted effects, which may be linked to circulating mitotane levels exceeding 20 mg/l (1, 23, 24). **Patients with mild kidney failure (BCC ≥ 60 cc/min) do not need specific dose adjustment but we do not have experience with more severe renal impairment.**

Since it is thought that mitotane needs metabolic activation to exert its action (2), measurement of mitotane (op' DDD) only may provide an incomplete figure. The only study that correlated levels of mitotane (op'-DDD) and its metabolites (op'-DDA and op'-DDE) to response in patients with advanced ACC found that the combined evaluation of op'-DDD and op'-DDA was useful to better characterize mitotane responders. Conversely, measurement of op'-DDE was useless (22). The role of mitotane metabolites should be addressed also in the adjuvant setting.

MITOTANE FOR ADVANCED ACC

Mitotane is part of the medical management of advanced ACC, either as monotherapy or combined with cytotoxic chemotherapy (1, 24). A recent prospective study provided novel information on mitotane pharmacokinetics comparing two different dosing regimens in patients with advanced ACC (25*). In 13 patients a low-dose starting regimen was used (a dose of 3 grams daily was reached after 12 days), while 27 patients were given a high-dose starting regimen (a dose of 6 grams daily was reached after 4 days and kept until day 14^{th}); further dose adjustments were guided by results of mitotane monitoring. The patients exposed to a greater mitotane dose reached peak plasma levels that were non-significantly higher compared with the low-dose group. The difference was significant only among patients not treated with chemotherapy. The frequency and severity of adverse events did not differ between the two groups. These data show that the rise in mitotane concentrations is slow and not fully predictable by the given mitotane dose; different variables factor on the amount of mitotane plasma concentrations and mitotane-related toxicity.

The study confirmed that mitotane is able to enhance hepatic protein synthesis/secretion. Levels of sex-hormone binding globulin (SHBG) increased during the trial and this may explain why free testosterone levels declined while total testosterone remained unchanged from baseline levels. Levels of thyroxine binding globulin (TBG) were also increased, although mitotane levels >14 mg/l were associated with low FT4 levels and unchanged TSH. These peculiar patterns of sex-steroid and thyroid hormones have been already recognized in a retrospective study in patients treated adjuvantly with mitotane (3). Mitotane use is also associated with increased levels of cortisol binding globulin (CBG) that may compound interpretation of serum cortisol levels during treatment (1, 3, 23, 24).

The issue of individual variability of mitotane concentrations has been recently addressed by D'Avolio et al. (26*), who investigated the potential impact of single-nucleotide polymorphisms (SNP) of CYP2B6 and ABCB1 genes, which are involved in mitotane metabolism, on the kinetics of mitotane levels in ACC patients. A retrospective analysis on 27 patients on post-operative adjunctive mitotane was performed and CYP2B6 and ABCB1 polymorphisms were genotyped and tested for association with plasma mitotane concentration. Patients with the GT/TT genotype showed higher mitotane plasma concentrations compared to patients with GG at 3 months and 6 months. Multivariate logistic regression analysis showed that only the CYP2B6 rs3745274GT/TT genotype was a predictor of mitotane concentrations ≥14 mg/L after 3 months of treatment. Thus, this study demonstrated a genetic basis for mitotane variability in blood.

The feasibility of a high-dose regimen in patients treated with mitotane monotherapy was also demonstrated in a prospective study from Institut Gustave Roussy, showing that 6 of 22 patients (27%) were able to reach a therapeutic mitotane level after 1 month of treatment (27). Thus, a high-dose strategy may be preferable when treating progressive ACC without the association of cytotoxic agents that limit patient compliance.

A major advancement in the field was the publication of the first randomized trial in advanced ACC, the FIRM-ACT trial, that compared two cytotoxic chemotherapy regimens, etoposide, doxorubicin, and cisplatin (EDP) versus streptozotocin, both combined with mitotane, as first-line treatment in patients with metastatic ACC (28**). The study demonstrated the superiority of EDP but since mitotane was part of both regimens it is difficult to recognize its contribution. Interestingly, in the 54 patients who had a mitotane level of 14 mg/l, or higher, at treatment start there was a trend toward increased OS as compared with the 212 patients who did not (HR for death, 0.76; 95% CI, 0.54 to 1.08; P=0.13). Further post-hoc analyses are ongoing to dissect the role of mitotane.

In advanced ACC patients, mitotane is often used in combination with other drugs even if we have limited knowledge of the all potential pharmacologic interactions between mitotane and different drugs. A phase II clinical trial done in Germany (29) showed that the association between mitotane and sunitinib is not sound since concomitant mitotane treatment resulted in rapid metabolism and reduced levels of sunitinib. A recent in vitro study demonstrated that mitotane induces CYP3A4 gene expression, explaining the drug–drug interactions caused by mitotane-enhanced CYP3A4 activity (30). Therefore, the concomitant administration of mitotane with anticancer drugs metabolized by CYP3A4 and CYP2B6 may result in sub-therapeutic plasma concentrations of these drugs due accelerated clearance. As discussed before, that mitotane induces CYP3A4 activity is also relevant to steroid replacement therapy of ACC patients.

Conclusions

Mitotane acts at the mitochondrial level in ACC cells interfering with cellular respiration and steroidogenesis. These effects contribute to the adrenolytic action of mitotane. Mitotane affects also metabolism of cortisol and a variety of drugs through induction of CYP3A4 activity.

Novel data in favor of adjunctive mitotane treatment have been published, but the level of evidence remains low in the absence of prospective studies. However, most experts recommend mitotane following extirpation of ACC in patients at high risk of recurrence (1, 23, 24). The

strategy of adjuvant treatment at our center is summarized in figure 3. In advanced ACC, the regimen EDP plus mitotane has been established as the reference treatment after publication of the first controlled study in this rare tumor, the FIRMACT trial (28). However, the specific contribution of mitotane to the activity of polychemotherapic regimens remains to be fully established.

Key points

- Mitochondria are the cellular targets of mitotane where it interferes with the respiratory chain activity and downregulates expression of steroidogenic enzymes.
- Mitotane induces CYP3A4 gene expression thus enhancing metabolic clearance of cortisol and a variety of drugs.
- It is still unclear which is the best strategy to give mitotane, even if a high-dose starting regimen provides elevated plasma concentrations in less time.
- Mitotane plasma concentrations should be kept higher than 14 mg/l during adjuvant treatment to attain a better outcome.
- The chemotherapic regimen EDP combined with mitotane is the current standard of care for advanced ACC.

Financial disclosure

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LEGENDS

Figure 1. Effects of mitotane on the mRNA expression of several steroidogenic enzymes. Black diamonds identify a definitive inhibitory effect while white diamonds indentify mixed effects (inhibition – no change; inhibition – stimulation) in different experimental conditions.

Figure 2. a) RFS of patients with mitotane levels ≥14 mg/l during follow-up (solid line) and patients with mitotane levels <14 mg/l (dashed line). (b) OS of patients with mitotane levels ≥14 mg/l during follow-up (solid line) and patients with mitotane levels <14 mg/l (dashed line). Adapted from Ref. 21.

Figure 3. The strategy of adjuvant mitotane treatment at San Luigi Hospital. In the prospective trial ADIUVO (http://www.adiuvo-trial.org) patients are randomized to mitotane or no treatment. The trial is endorsed by the European Network for the Study of Adrenal Tumors (ENS@T) and is currently recruiting at different European and North American centers.