Mechanisms of Mitotane Action in Adrenocortical Cancer

Subjects: Oncology

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Mitotane is the only approved drug for the treatment of advanced adrenocortical carcinoma and is increasingly used for postoperative adjuvant therapy. Mitotane action involves the deregulation of cytochromes P450 enzymes, depolarization of mitochondrial membranes, and accumulation of free cholesterol, leading to cell death.

mitotane adrenocortical carcinoma cytochromes P450 enzymes Mitochondria-associated membranes

1. Introduction

Mitotane, 1,1-(o,p′-Dichlorodiphenyl)-2,2-dichloroethane (o,p′-DDD), commercially available as Lysodren® (HRA Pharma Rare Diseases, Paris, France), is a parent compound of the insecticide dichlorodiphenyltrichloroethane (DDT). o,p′-DDD is metabolized by the mitochondria of adrenal cells in DDE (1,1-(o,p′-Dichlorodiphenyl)-2,2 dichloroethene) and DDA (1,1-(o,p′-Dichlorodiphenyl) acetic acid) through α-hydroxylation and β-hydroxylation, respectively. In addition, the unstable precursor of DDA, o,p′-dichlorodiphenyl acyl chloride (DDAC), obtained through cytochrome P540 (CYP450), could covalently bind to mitochondrial macromolecules of adrenal cells or can be metabolized by CYP2B6 in the liver or intestine, reducing its bioavailability [1]. Mitotane is the reference drug for the treatment of advanced adrenocortical carcinoma (ACC) either alone or in combination with chemotherapy [2][3] and is increasingly used for postoperative adjuvant therapy [1][2][3][4][5].

Although mitotane can exert its effects on the gonads and pituitary gland [6][7][8][9], it acts primarily on the adrenal cortex leading to cell destruction and impairment of steroidogenesis [10][11][12]. Indeed, mitotane produces dose-related cellular toxicity causing the rupture of mitochondrial membranes mainly on the zona fasciculata and reticularis, whereas a minimal effect on the zona glomerulosa has been observed [13]. This differential action explains why aldosterone secretion is less affected by mitotane treatment [14][15]. It is generally accepted that circulating levels of mitotane should be maintained between 14 and 20 mg/L (approximately 40–60 µM), the therapeutic window, to obtain the anti-tumoral effect while avoiding severe neurological toxicity [3][16]. Indeed, several retrospective analyzes have shown that mitotane blood concentrations ≥14 mg/L are associated with a disease response in both advanced and adjuvant ACC treatment [17][18][19][20][21][22]. The upper limits are more uncertain; in fact, central neurological toxicity has been more frequently associated with elevated mitotane concentrations (>20 mg/L), but mild symptoms can be observed even with lower plasma levels [17][23]. Studies, however, have suggested that inhibition of steroid secretion could be obtained even with lower mitotane levels [24]

[25]. Mitotane accumulates in lipoproteins and is stored in adipose tissue, although little is known about how this distribution affects its effectiveness [26].

2. Mitotane Effects on Mitochondrial Membrane and Gene Expression

Mitotane seems to act selectively on the adrenal cortex affecting steroidogenesis. This specificity for the adrenal cortex could be related to the massive presence in these cells of enzymes involved in steroidogenesis and/or cholesterol metabolism that could interact directly with mitotane (**Figure 1**). Indeed, mitotane shares characteristics with other endocrine disruptors and may affect steroidogenesis by binding to steroid receptors, mimicking the action of steroids [27]. A binding between mitotane and cytochrome P450 has been directly observed [28][29][30]. Interestingly, this interaction inhibits CYP11A1-mediated metabolic transformation regardless of the presence of the CYP11A1 substrate or its inhibitor. This result may indicate that either CYP11A1 is not the mitotane activator or that mitotane activation is not required to destroy CYP enzyme function. Indeed, the formation of adducts can affect the endogenous function of critical target proteins and thus directly causes toxicity or binds to non-essential proteins and thus constitutes an exposure biomarker [31]. Similar behavior was observed in murine corticosterone-producing Y1 cell line [28]. Furthermore, mitotane-induced protein adducts could also explain the altered transcriptomic profile, with varying degrees of post-translational modifications, identified by Stigliano et al. [12].

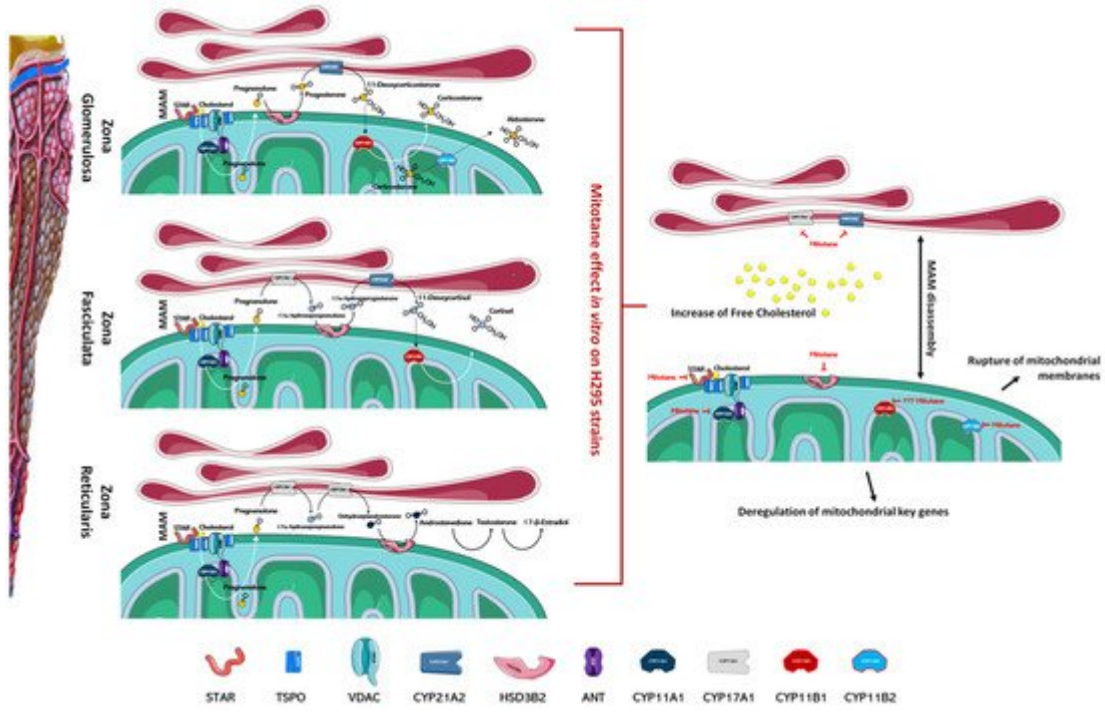

Figure 1. Mitotane impairs the function of the adrenal cortex. In the left part of the figure, the different zones of the adrenal cortex are schematized; the main enzymes involved in the biosynthesis of steroid hormones are also indicated. As depicted in the right part of figure, mitotane action, identified by in vitro experiments, involves several mechanisms ranging from the deregulation of mitochondrial key genes at a transcriptional and functional level, to the MAMs dissociation, the rupture of mitochondrial membranes, and altered cholesterol transports/metabolism.

Mitotane action for each enzyme is indicated by a red mark. Figures have been created modifying an image set from Servier Medical Art (SMART) http://smart.servier.com/ (19 July 2021).

Several articles have reported that mitochondria are the organelles primarily involved in mitotane susceptibility in adrenal cells. This action involves several mechanisms ranging from the deregulation of mitochondrial key genes to the rupture of mitochondrial membranes (**Figure 1**). Mitotane affects mitochondrial enzymes at a transcriptional and functional level and significantly decreases the expression of the protein that transports cholesterol into mitochondria and of its related gene *STAR* [26][32][33]. Inside of mitochondria, cholesterol is converted to pregnenolone by CYP11A1 and, as indicated previously, mitotane mediates functional and transcriptional CYP11A1 inhibition [26][32][33][34][35][36][37]. Further, mitotane-related downregulation of steroidogenic enzymes *HSD3B2*, encoding for 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase, and *CYP21A2*, encoding for steroid 21-hydroxylase, was also observed [33][38]. Contrasting results were obtained for the *CYP11B1* gene, encoding for the enzyme 11b-hydroxylase, which catalyzes the transformation of 11-deoxycorticosterone and 11-deoxycortisol into corticosterone and cortisol, respectively [32][38][39][40][41].

Mitotane blocks the ACTH/cAMP-related signaling, although contrasting results due to specific human cell models have been observed. In particular, H295A are non-responsive, whereas H295R respond to this hormone depending on subclones and culture conditions [42]. The response of the H295 progenitor cell line is not so clear; it is often indicated as ACTH-unresponsive [42] but probably follows the same behavior of H295R cells. Indeed, Lin et al. showed that H295 responds to increasing ACTH concentration by increasing cortisol secretion and that mitotane was able to completely abolish this response [32].

Mitotane could also affect the angiotensin II/K+ related signaling principally responsible for CYP11B2 transcription. All H295R strains, including the subclone HAC15, respond to this molecular signaling pathway, in contrast to H295A, which are selected as not responder cells. No indication of angiotensin II/K+ signaling was obtained for the H295 progenitor cell line [42]. Although all studies agree on the blocking action of mitotane on corticosteroid synthesis, conflicting results in molecular pathways and in the deregulation of specific genes or enzymes could support the hypothesis that specific cell line characteristics and variable experimental conditions have an important impact on mitotane action and should be carefully considered for a meaningful assessment of in vitro studies on mitotane.

3. Physiological Regulation of Cholesterol Uptake, Synthesis, and Steroidogenesis and the Proposed Mitotane Effect/Mechanism of Action

Mitochondria-associated membranes (MAM) are reversible contact points between the mitochondria and the endoplasmic reticulum (ER) membrane and are involved in the mitochondrial import of certain lipids, such as cholesterol. The presence of several enzymatic targets responsive to mitotane in mitochondria and MAM caused a progressive alteration in mitochondrial structure and the number of normal mitochondria when H295R were exposed to mitotane (**Figure 1** and **Figure 2**). In addition, a more punctiform pattern, as a sign of mitochondrial

fragmentation, was frequently observed [38][43]. Further, mitotane exposure alters the MAM integrity, reducing the interactions between mitochondria and ER in H295R [36]. These results could be related to a progressive depolarization of the mitochondrial membrane, also due to the functional block of COX enzymes, with consequent interruption of the respiratory system and MAM disassembly [36][38]. Sterol O-acyltransferase enzymes, SOAT1 and SOAT2, are located within MAM and catalyze cholesteryl esters formation from cholesterol. Sbiera et al. identified SOAT1 as the key molecular target of mitotane and showed a correlation between SOAT1 expression and the outcome of adjuvant mitotane treatment [44], whereas Lacombe et al. found that SOAT1 expression is a prognostic marker in combination with the Ki67 index [45]. Unfortunately, the hypothesis that SOAT1 expression could be a clinically useful marker for predicting treatment response to mitotane has not been confirmed by further studies [46] [47]. Weigand et al. retrospectively analyzed data of 231 patients with ACC treated with mitotane in 12 reference centers and did not find any significant differences between tumors with high or low SOAT1 expression in terms of recurrence-free survival (in 158 patients treated with adjuvant mitotane), progression-free survival (in 73 patients with advanced ACC), or disease-specific survival (in both settings) [47].

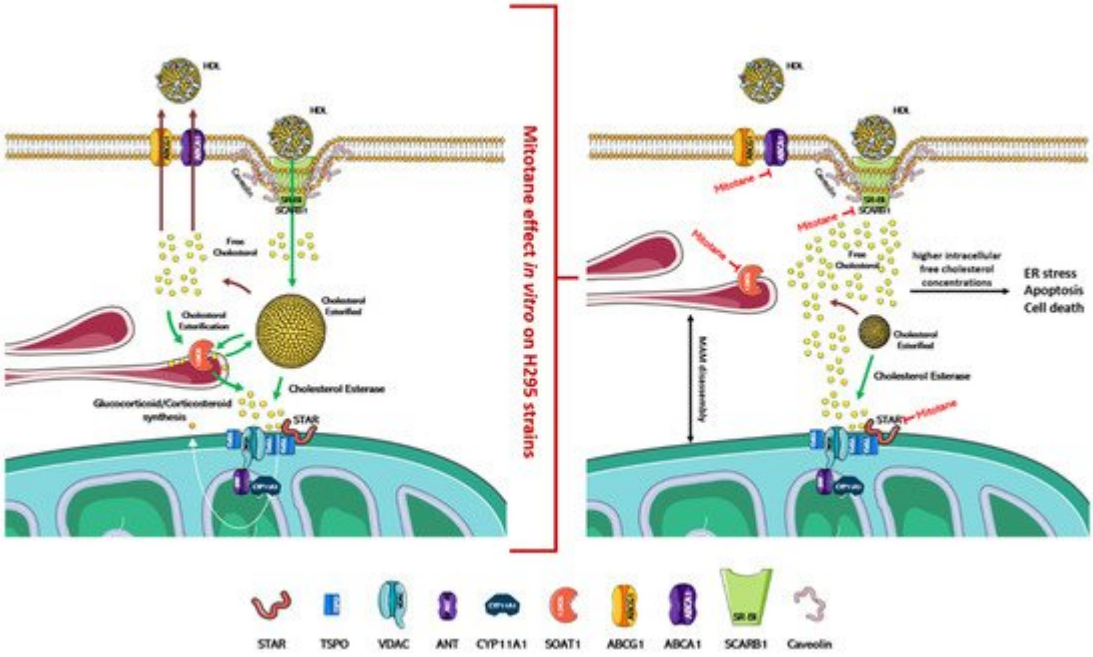

Figure 2. Physiological regulation of cholesterol uptake, synthesis, and steroidogenesis and proposed mitotane effect/mechanism of action. In the left part of the figure is indicated the physiological mechanism that regulates the absorption/synthesis of cholesterol and steroidogenesis. As depicted in the right part of the figure, mitotane induces in vitro the dissociation of MAMs and the blockade of cholesterol transport/synthesis and steroidogenesis. Accumulation of free cholesterol in cells causes ER stress, apoptosis, and cell death. The action of mitotane for each enzyme is indicated by a red mark. Figures were created modifying an image set from SMART http://smart.servier.com/ (19 July 2021).

In vitro, mitotane induces ER stress through inhibition of SOAT1, which leads to the blockade of cholesterol synthesis and steroidogenesis, and this accumulation of free cholesterol rapidly becomes toxic to the cells (**Figure 2**) [44][48]. Furthermore, mitotane in H295R subclones reduces the expression of ABCA1, which is involved in the

cellular efflux of cholesterol [49], and of *SCARB1*, which encodes for scavenger receptor B1 (SR-BI), the most important transporter for adrenal cholesterol uptake [33][50]. The adrenal cortex has critical enzymes and substrates necessary for ferroptosis, a form of iron-dependent cell death associated with increased lipid peroxidation. Curiously, despite the strong induction of lipid peroxidation, mitotane does not induce ferroptosis [51][52]. Since mitotane increases free cholesterol in cells and oxysterols, such as 27-hydroxycholesterol, which could reduce this process [53], the cholesterol metabolism could be an interesting druggable pathway to counteract mitotane resistance in ACC. On these bases, the introduction of LXRα and PCSK9 inhibitors as future therapeutic approaches could be a promising tool to reduce mitotane resistance and/or to optimize its therapeutic dose [33][53]. In the adrenal gland, the role of LXRα and its oxysterol ligands are critically important in the fine regulation of cholesterol efflux since the excess free cholesterol in cells is converted into oxysterols through the action of enzymes, such as CYP27A1. Pharmacological inhibition of LXRα significantly reduces the expression of the cholesterol efflux pump (ABCA1 and ABCG1) and is accompanied by higher intracellular free cholesterol concentrations, ER stress, apoptosis, and cell death markers expression. This effect is complementary to mitotane-induced lipotoxicity, and, using a combined therapeutic approach, lower doses of mitotane can be expected to be used, resulting in reduced toxicity [53].

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