BIOCHEMICAL MECHANISM OF THE o,p′-DDD EFFECT ON THE ADRENAL CORTEX

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o,p′-Dichlorodiphenyldichloroethane (o,p′-DDD, mitotane) is used in the treatment of adrenocortical cancer and Cushing’s disease. This medicine induces numerous biochemical changes in the adrenal cortex, as well as disorder in the mitochondrial structure. Therewith, the level of produced corticosteroid hormones is significantly reduced. One of the possible causes can be a decrease in the NADPH level due to inhibition of the activity of its reduction system and increased NADPH consumption during the glutathione reduction catalyzed by glutathione reductase. o,p′-DDD is partially metabolized in the adrenal glands, and the main metabolite (in terms of quantity) is o,p′-dichlorodiphenylacetic acid. However, attempts to find a physiologically active component among metabolites were unsuccessful. The most pronounced changes caused by o,p′-DDD were found in the mitochondria of the adrenal cortex. The respiration at the level of IV and I complexes is suppressed, the protein content of these complexes decreases. The phospholipid composition of the tissue altered and the concentration of diphasphatidylglycerol, the most important component of mitochondrial membranes, decreased. In our opinion, o,p′-DDD, owing to its high lipophilicity, accumulates in the mitochondria membranes and causes conformational disorder followed by disorder in mitochondrial functions. It was shown that o,p′-DDD acts as an inhibitor of acyl-CoA-cholesterol acyltransferase (ACAT, SОАТ1). Therefore, adenocorticocytes accumulate free cholesterol, causing endoplasmic reticulum stress, mitochondrial swelling and caspases activation. Increased apoptosis leads to a decline in adrenal function and to a decrease in weight of adrenal glands.

K e y w o r d s: adrenal cortex, steroidogenesis, mitotane, chloditane, glutathione, mitochondria, endoplasmic reticulum stress, apoptosis.

o,p′-Dichlorodiphenyldichloroethane (o,p′-DDD) was used for many decades to treat adrenocortical cancer and Cushing’s syndrome. In medical practice it is used under the name of Mitotane (Lisodren), in the USSR it was produced under the name Chloditane. Various aspects of the biological activity of o,p′-DDD have been thoroughly studied in the Institute of Endocrinology and Metabolism. The obtained results were summarized and analyzed in the monograph by V. P. Komissarenko and A. G. Reznikov [1]. In this review we present findings on the mechanism of action of o,p′-DDD that have been reported in the years since.

The mechanism of the o,p′-DDD effect on the adrenal cortex has been studied since its adrenocorticoletic effect was found [2]. Even early studies showed that the effect of o,p′-DDD is species-specific. Thus, adrenal glands of rats, mice, guinea pigs and sheep were found to be o,p′-DDD resistant, and only in humans and dogs o,p′-DDD inhibited the secretion of corticosteroids and causes atrophy of the adrenal cortex. However, attempts to inhibit steroi-
Some experiments had no success. It is known that o,p‘-DDD is virtually insoluble in water (0.1 mg/l at 25 °C; 0.3 μM), and adding of o,p‘-DDD in powder to the incubation medium in some experiments had no success. Important results that shed light on the conditions for realization of o,p‘-DDD action in vitro were obtained by V. I. Kravchenko [3]. Blood plasma containing a propylene glycol solution of o,p‘-DDD was used in the reported work. It was found that at incubation of dog adrenal slices under these conditions, synthesis of corticosteroids from endogenous precursors, as well as from added progesterone did not alter. However, in the experiments with dog adrenal homogenate, a clear decrease in the corticosteroid production was observed [3]. The penetrating o,p‘-DDD into cells might be a very complex process, so the incubation of the slices appears to be not sufficiently accurate method in studying the action of o,p‘-DDD. A successful attempt to inhibit the corticosteroids secretion by o,p‘-DDD was reported in [4]. The authors perfused the dog adrenal glands with a solution of o,p‘-DDD in Krebs-Ringer-bicarbonate medium or in blood plasma. It was established that the adrenal cortex did not respond to ACTH, added to the perfused solution, by increased corticosteroid production. Inhibition of steroidogenesis during the incubation of slices in the o,p‘-DDD containing medium was not observed.

In dogs treated with o,p‘-DDD, a variety of biochemical changes in the cortical tissue and atrophy of the gland cortical layer were observed. Thus, a decrease in the activity of enzymes involved in NADP reduction (a cofactor of hydroxylase reactions of steroidogenesis) was established [5]. NADPH serves as a hydrogen donor for hydroxylations reactions – the most important reactions of steroidogenesis. It is also a cofactor of glutathione reductase, which is involved in maintaining the necessary level of reduced glutathione in cells. In the adrenal tissue of dogs receiving o,p‘-DDD, a considerable decrease in the content of SH-groups was observed [6]. Under these conditions the glutathione reductase activity substantially increased. This increase was observed already 24 h after a single feeding with o,p‘-DDD at a dose of 50 mg per kg of animal weight [7]. Such increase in the activity of glutathione reductase might be a compensatory response. In vitro, o,p‘-DDD also activated glutathione reductase of the adrenal glands, but not of the kidneys and liver [8].

It is well known that glutathione is involved in neutralizing peroxides, reactive oxygen species and foreign compounds. The ability to neutralize foreign compounds determines, to a large extent, the drug efficacy and the development of tumor resistance to pharmaceutical agents. These findings prompted us to study how o,p‘-DDD affects glutathione-S-transferase (EC 2.5.1.18) activity in animals who are sensitive or resistant to o,p‘-DDD adrenocortico-lytic action. Feeding dogs with o,p‘-DDD caused a decrease in the enzyme activity in the adrenal glands, but did not change the enzyme activity in the liver. Inhibition of glutathione-S-transferase activity in the adrenal glands of dogs receiving o,p‘-DDD may be account for the adrenocortico-lytic effect of the drug. In guinea pigs, the enzyme activity increased in both the adrenal glands and the liver [9]. In vitro o,p‘-DDD markedly inhibited the activity of glutathione-S-transferase in dog adrenal glands and less markedly in the adrenals of guinea pigs. In rats receiving o,p‘-DDD, the activity of glutathione-S-transferase in the adrenal glands also decreased, but increased in the liver [10]. The level of reduced glutathione in these tissues changed in the opposite way – increased in the adrenal glands and decreased in the liver. This is the typical response of liver tissue to xenobiotics.

Since treatment of adrenocortical cancer with o,p‘-DDD does not always give positive results, some researchers have tried to find biochemical predictors of the drug efficacy. Thus, ribonucleotide reductase (EC 1.17.4.1) was considered as such a predictor [11]. This enzyme catalyzes the synthesis of deoxyribonucleotides essential for DNA synthesis. The enzyme contains two subunits encoded by different genes. The pronounced expression of RRM1 gene in patients is associated with shortening of remission and life expectancy. Thus, in patients with low level of RRM1 expression, mitotane treatment prolonged the remission period. In patients with high level of RRM1 expression such effect was not observed. In vitro, mitotane caused a significant increase in RRM1 transcription in SW13 cells, which are resistant to mitotane, but not in mitotane susceptible H295R cells. Moreover, the suppression of RRM1 by specific microRNAs sensitized SW13 cells to mitotane. The authors suggested that RRM1 may serve as a biomarker enabling to predict the tumor response to mitotane treatment. A deeper analysis of biomarkers for adrenocortical cancer sensitivity/resistance to drugs was reported in [12]. The results showed that mitotane and cisplatin are effective in treatment of patients with low level of proteins.
RRM1 and ERCC1 (DNA excision repair protein). CYP2W1 was also considered as a biomarker [13]. The authors found that CYP2W1 expression was high in the majority of adrenal tumors both benign and malignant. Moreover, the level of immunoreactive CYP2W1 in tumors is associated with hormone activity, a more differentiated phenotype, and in cancer, with a better response to mitotane treatment. These findings allow considering CYP2W1 as a possible marker to prognose tumor response to mitotane used as an adjuvant or palliative therapy after surgery. The activity of topoisomerase IIα and thymidylate synthase as prognostic criteria was analyzed in [14]. The activity of these enzymes in tumor tissue was found not to be associated with the effect of mitotane.

Metabolism of o,p'-DDD incubated with dog adrenal mitochondria, microsomes and cytosol was studied [15]. In the presence of NADPH and system of its reduction, mitochondria converted o,p'-DDD into five different compounds, among which o,p'-dichlorodiphenylylacetic acid was identified. The metabolism was sharply inhibited when the incubation was carried out in a CO atmosphere, or in the absence of NADPH in the medium. o,p'-DDD was not metabolized in cytosol and microsome fractions. The authors suggested that the ability of metabolites to covalently bind to mitochondria proteins determines the adrenocorticotolytic activity of o,p'-DDD [16]. Incubation of dog adrenal homogenate with 125I-labeled o,p'-DDD showed that the radioactivity was mainly associated with proteins, and the phospholipid fraction contained about 17% of the total radioactivity [17]. After hydrolysis of the complexes, the only metabolite of o,p'-DDD was found to be chlorophenyl-1-iodophenylacetic acid. After incubation of the dog, bovine and human adrenal homogenates, the bound o,p'-DDD metabolites were separated by electrophoresis [18]. The electrophoreograms were similar for all homogenates. Among the radiolabeled proteins, cytochrome P450scs was detected by Western blotting. The hypothesis that o,p'-dichlorodiphenylylacetic acid (o,p'-DDA) is the active metabolite of o,p'-DDD was tested [19]. The authors studied the effect of o,p'-DDA on H295R and SW13 cell lines. o,p'-DDA, unlike o,p'-DDD, did not inhibit cell proliferation, nor decrease gene expression, respiration, nor cause oxidative stress or apoptosis. During incubation of the cells with o,p'-DDA, its concentration in the medium, in contrast to o,p'-DDD, did not alter suggesting that o,p'-DDA did not enter the cells. o,p'-DDA was not detectable in adrenal tissue of o,p'-DDD-treated patients indicating that there was no cellular o,p'-DDA uptake or its in situ formation [19].

Histologic and electron microscopic changes in the dog adrenal cortex occurring after o,p'-DDD administration were described in a number of research [20-22]. Electron microscopy analysis demonstrated that adrenal mitochondria swelled in 2-4 h after a single administration of o,p'-DDD to dogs. o,p'-DDD was found to affect endoplasmic reticulum, to lead to myeloid-like structure formation, as well as an increase in the number and size of autolysosome structures [20, 21]. Analysis of H295R and SW13 adrenocortical cancer cells exposed to o,p'-DDD by electron microscopy revealed dose- and time-dependent changes in mitochondria structure [23]. Swelling and disruption of cristae, formation of myelin-like structures on the inner mitochondrial membrane as well as cytoplasmic vacuolization were observed. Increasing of o,p'-DDD concentration led to a decrease in the total number of mitochondria. Mitochondria swelling and degeneration were also observed in the experiments on culture of Fang-8 cells exposed to o,p'-DDD (Fang-8 cells derived from estrogen-producing adrenal tumor and retained steroidogenic activity) [24]. In vitro o,p'-DDD caused a drastic inhibition of mitochondrial respiration in the liver and kidneys of dogs and guinea pigs [25].

Development of stable culture of adrenal tumor cells played an important role in further research of the mechanism of o,p'-DDD action – many researchers began to use them in the studies. o,p'-DDD (10-40 μM) inhibited basal and cAMP-activated hydrocortisone secretion by H295 cells, but did not cause their death [26]. It inhibited the basal expression of StAR and P450c17 proteins and decreased the expression of mRNA StAR, CYP11A1 and CYP21. It was found that o,p'-DDD has a stimulatory effect on CYP11B1. The addition of cAMP to control samples elevated the expression of steroidogenic genes. o,p'-DDD (40 μM) almost completely neutralized the effect of cAMP. The authors concluded that o,p'-DDD can inhibit corticosteroid biosynthesis via non-specific interaction with transcription of steroidogenic enzymes. Gene expression in H295R cells treated with o,p'-DDD at concentrations that inhibit steroidogenesis but do not affect cell viability was studied [27]. Isoalted RNA was further analyzed using microarrays. Compared to control, differences in the expression of
117 genes were found. Three steroidogenesis regulatory genes HS3DB1, HS3DB2 and CYP21A2 were underexpressed, and four genes GDF15, ALDH1L2, TRIB3 and SERPINE2 were overexpressed. A decrease in the mRNA level of cytochromes CYP11A1 and CYP17A1 in H295R cells in the presence of o,p'-DDD was shown in the study [28]. o,p'-DDD was found to cause a decrease in the level of mRNA protein kinase A regulatory subunit (PRKAR1A). These changes explain the molecular mechanisms of inhibition of steroidogenesis by o,p'-DDD.

Qualitative and quantitative alterations in the mitochondrial morphology and function in H295R and SW13 cells were evaluated in [23]. The accumulation of o,p'-DDD and o,p'-DDE (o,p'-dichlorodiphenyldichloroethylene – metabolite of o,p'-DDD) in H295R cells was found to be dependent on the concentration (directly proportional) in the medium (10-100 μM). The number of cells decreased proportionally to concentration and time. Such decrease may result from the combined effect on viability and proliferation. The IC\textsubscript{50} for these parameters ranged from 10 to 20 μM. The toxic effect of o,p'-DDD on SW13 cells was also established. Quantification of apoptosis by annexin V showed that exposure to o,p'-DDD at doses of 30 and 50 μM for 48 h led to an increase in the percentage of apoptotic cells. The level of caspase-3 and -7 increased in the cells. The increased caspase-3 and -7 activities in these cells were also shown by other researchers [28]. The influence of o,p'-DDD on apoptotic DNA fragmentation in vitro was analyzed in our work [29]. It was shown that apoptosis significantly increased during incubation of the adrenocortical adenoma slices in the presence of 50 μM o,p'-DDD. The effects of o,p'-DDD are particularly pronounced in the conditions of activation of the adrenal cortex function [30]. Even at a concentration of 5 μM, o,p'-DDD prevents Ca ions penetration into cells triggered by an increase in the medium K+ level. o,p'-DDD significantly inhibited the activation of protein, RNA and DNA synthesis under conditions of activation of aldosterone synthesis by increased K+ level.

The mitochondrial function was monitored by measuring the membrane potential using JC-1 dye. o,p'-DDD caused a more pronounced decrease in the membrane potential compared to effect of well-known ionophore valinomycin. Oxygen consumption by mitochondria isolated from H295R cells exposed to o,p'-DDD for 48 h was determined. Even concentration of 10 μM led to a significant decrease in respiration in the presence of succinate. Inhibition increased up to 82% at a concentration of 50 μM. Western blot analysis showed that o,p'-DDD reduced the expression of the mitochondrial anion channel VDAC in the outer membrane, increased the expression of caspase-3 but did not affect the level of succinate dehydrogenase (complex II) in the mitochondrial inner membrane [23]. The effect of o,p'-DDD on respiratory chain was studied on cultured H295R and SW13 cells derived from human adrenocortical carcinoma [31]. It was shown that o,p'-DDD at a concentration of 50 μM inhibited respiration 2-fold in both cell lines at the stage of complex IV (cytochrome c oxidase). The activity of complex II (succinate-ubiquinone oxidoreductase) remained unchanged. Complex III (ubiquinol-cytochrome c oxidoreductase) was inhibited slightly and only in SW13 cells. The IC\textsubscript{50} for the IV complex was found to be ~ 67 μM. It is known that cytochrome oxidase includes 13 subunits; three of them are encoded by the mitochondrial genome, the rest – by nuclear. Mitochondrion : nuclear subunits ratio in transcripts reduced in H295R cells treated by o,p'-DDD by 70%. Similar results were obtained in experiments with SW13 cells. Protein analysis revealed that exposure for 48 h reduced the level of complexes IV and I by 45-70%, but did not alter the level of complexes II and III [31].

The above data demonstrate that mitochondria are likely to be the main intracellular target for the o,p'-DDD action. It is mitochondria where the most important reactions of respiration and oxidative phosphorylation as well as steroidogenic reactions occur. Interaction between mitochondria and endoplasmic reticulum (ER) was reported to play an important role in steroidogenesis [32]. This interaction occurs in the specialized contact cites and results in the formation of specific subcellular domains called mitochondrial-associated membranes (MAMs). This coupling plays an important role in some cellular processes, in particular, in Ca\textsuperscript{2+} transport and apoptosis [33]. Thus, MAMs may be considered as functional adapters of changes in cell homeostasis. It was shown in [34] that FATE1, a cancer-testis antigen, plays an important role in the regulation of the ER - mitochondria distance [34]. FATE1 is expressed in adrenocarcinoma cells under the control of the transcription factor SF1 and increases the distance between ER and mitochondria, reducing mitochondrial Ca\textsuperscript{2+} uptake. FATE1 also decreases sensitivity to pro-apoptotic effects and to o,p'-DDD [34].
Steroidogenesis occurs mainly on mitochondrial membranes, the main components of which are cholesterol and phospholipids [35]. The adrenal gland is characterized by a high content of 7,10,13,16-docosatetraenoic acid (a trivial name adrenic acid) in phospholipids. The phospholipid composition of the adrenal glands of humans and dogs is very close and differ only in the sphingomyelin level which was found to be significantly higher in humans [36]. In dogs treated with o,p′-DDD (50 mg/kg) the phospholipid composition of the adrenal cortex was shown to be altered. The level of phosphatidylserine, phosphatidylinositol and sphingomyelin increased, whereas the level of diphasphatidylglycerol and lysophosphatidylethanolamine decreased. Such decrease in the diphasphatidylglycerol level is associated with the destruction of mitochondria caused by o,p′-DDD [37]. It is known that diphasphatidylglycerol (cardiolipin) is an important constituent of the inner mitochondrial membranes [38]. The formation of a cholesterol complex with cytochrome P450 (which cleaves the cholesterol side chain to form pregnenolone) appeared to depend on the phospholipid composition of the medium, and cardiolipin is the most active phospholipid [39].

A direct interaction of o,p′-DDD with membranes was studied on unilamellar membrane system prepared from cholesterol and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine) [40]. o,p′-DDD constituted 20 molecular % of phospholipids. It was found that o,p′-DDD and its main metabolite dichlorodiphenyl-lactic acid (o,p′-DDA) bind to lipid membranes anchoring into the lipid-water interface. o,p′-DDD, but not o,p′-DDA, directly causes a disruption to the bilayer structure leading to increased membrane permeability for polar molecules. We studied the effect of o,p′-DDD on the process of mitochondria swelling in vitro [41]. Adding o,p′-DDD to the medium was found to increase dramatically the rate and degree of the adrenal mitochondria swelling. The nature and value of the changes depend on the o,p′-DDD concentration in the conditions of constant concentration of mitochondrial protein. The peculiarity of the o,p′-DDD-induced swelling is the inverse temperature-degree dependence: with increasing temperature, the degree of swelling decreases. These findings may indicate that weak bonds (hydrophobic or Van der Waals) are formed between o,p′-DDD and the mitochondrial lipid components. These bonds readily dissociate as the temperature rises. The significance of the membrane structures in the effects of o,p′-DDD was confirmed by the results of Jacobi et al [42]. The authors studied the effect of o,p′-DDD on human erythrocytes in vitro. At the therapeutic doses of o,p′-DDD, phosphatidylserine translocation in erythrocyte membranes from the inner layer into the outer layer occurred. Under these conditions the cellular Ca2+ level substantially increased, hemolysis was enhanced. In the absence of Ca2+, inhibition of translocation of phosphatidylserine, determined by annexin V binding, was observed. Moreover, o,p′-DDD inhibited the Ca2+-induced shrinkage of erythrocytes.

In 1994, at the European Congress on Endocrinology, we hypothesized that adrenocorticocty membranes are the main locus of the o,p′-DDD action. Dissolving in the lipid phase of membranes, it disrupted their organization, and changes in the membrane physico-chemical properties led to the cell damage [43]. The possible sequence of events was suggested to be as follows: 1. Accumulation of o,p′-DDD in membranes. 2. Damage to the membrane functions (ion pumps, electron transport, function of membrane-bound enzymes). 3. Activation of peroxidation processes. 4. Impairment of synthesis and function of macromolecules by products of peroxidation (Fig.).

The discovered inhibitory effect of o,p′-DDD on sterol O-acyltransferase (EC 2.3.1.26), often referred to as ACAT (acyl-CoA cholesterol acyltransferase) or SOAT [44], has drawn a lot of attention. In adrenal tissue this enzyme converts cholesterol into its esters. There are two isoforms of the enzyme that differ in distribution and function. SOAT1 is present in adrenal, kidney and macrophages. Some inhibitors (PD132301-2 (or ATR-101) is the most studied) of this enzyme, developed for treatment of atherosclerosis, induce degeneration of adrenal cortex [45]. This effect was observed in dogs; however it was not found in rats and mice. The study of inhibition of ACAT significantly improved and deepened the understanding of the mechanism of o,p′-DDD adrenocorticolytic activity.

In vitro, in H295R cells, ATR-101 induced mitochondria hyperpolarization, increased the ROS formation within 1 h of exposure [46]. ATR-101 treatment of H295R cells led to the accumulation of free fatty acids and cholesterol that induces ER stress [47]. In so doing the increased caspase 3/7 activity and activation of apoptosis, assessed by terminal deoxynucleotidyl transferase nick end labe-
The scheme of mitochondrial processes affected by o,p'-dichlorodiphenyldichloroethane (o,p'-DDD, mitotane, chloditane). A – adrenodoxin, AR – adrenodoxin reductase, Cyt P450 – cytochrome P450_5α, I-IV – mitochondrial respiratory chain enzymes, Q – ubiquinone

lining (TUNEL assay), were observed. ATR-101 also caused the unfolded protein response resulting in ER stress. Treatment of dogs with ATR-101 decreased the formation of corticosteroids and induced adrenocorticocytes apoptosis, followed by a decrease in the adrenal cortex. The authors suggested that the use of ATR-101 (the ACAT inhibitor) may be promising in the treatment of adrenalcortical cancer [47]. In H295 cells, o,p'-DDD dramatically increased the expression of reticular stress marker CHOP (transcription factor encoded by the DDIT3 gene) [44]. o,p'-DDD also activated two preceding stress factors XBP1 and eIF2α. In adrenal cancer cells, the expression of ACAT varies and this can define the o,p'-DDD susceptibility of these cells. Since the endoplasmic reticulum stress can be activated by various compounds, the potential synergy between o,p'-DDD and proteasome inhibitors: MG132, bortezomib
and carfilzomib was studied [48]. The expression of mRNA and proteins, markers of endoplasmic reticular stress, was evaluated on H295 cells. Synergism between o,p'-DDD and proteasome inhibitors was shown. Proteasome inhibitors can be considered as promising agents for the adrenocortical cancer treatment in combination with o,p'-DDD.

The data presented in the review shows that o,p'-DDD, being a highly lipophilic compound, interacts with the adrenal membrane structures. This accumulation causes a disorder in function of mitochondrial membranes and endoplasmic reticulum, resulting in apoptosis and adrenocorticoctye death. Understanding the mechanism of o,p'-DDD adrenocorticoctytic action enables researchers to find possible ways to improve efficacy of treatment of adrenocortical cancer and Cushing's disease.

**БІОХІМІЧНИЙ МЕХАНІЗМ ДІЇ o,n'-ДДД НА КОРУ НАДНИРКОВИХ ЗАЛОЗ**

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В огляді наведено дані щодо біохімічного механізму дії o,n'-дихлордифенілдихлоретану (o,n'-ДДД, мітотан), який використовується для лікування раці кори надніркових залоз і хвороби Кушинга. Під впливом препарату виникають численні біохімічні зміни в корі надніркових залоз і порушується структура мітохондрій. При цьому істотно знижується утворення коптостероїдних гормонів. Однією з можливих причин цього може бути зниження рівня NADPH через пригнічення активності систем його видновлення і підвищеної витрати для відновлення глутатіону глутатіонредуктазою. У надніркових залозах o,n'-ДДД частково метаболізується його основним метаболітом - o,n'-дихлордифенілнітрат (з точки зору кількості). Однак спроби знайти серед метаболітів фізіологічно активний компонент не мали успіху. Найвираховані зміни, спричинені o,n'-ДДД, здійснено в мітохондріях коркового шару надніркових залоз: пригнічується дихання на рівні IV і I комплексів, знижується вміст протеїнів цих комплексів, змінюється фосфоліпідний склад тканини і знижується вміст дифосфатидилгліцеролу – найважливішого компонента мембран мітохондрій. На нашу думку, o,n'-ДДД завдяки високій ліпофільністі накопичується в мембранах мітохондрій і зумовлює їх конформаційне порушення з подальшим порушенням функції. Встановлено, що o,n'-ДДД є інгібітором аціла-СоA-холестерол-ацилтрансферази (АХАТ, SOAT1). Через це в адренокортикотоцитах накопичується вільний холестерол, виникає стрес ендоплазматичного ретікулума, розвивається набухання мітохондрій і активуються каспази. Посилення апоптозу призводить до зменшення функції залози і зниження її маси.

**КЛЮЧОВІ СЛОВА:** кора надніркових залоз, стероїдогенез, мітотан, хлордан, глутатіон, мітохондрії, стрес ендоплазматичного ретікулума, апоптоз.

**БІОХІМІЧЕСКИЙ МЕХАНИЗМ ДЕЙСТВИЯ o,n'-ДДД НА КОРУ НАДПОЧЕЧНЫХ ЖЕЛЕЗ**

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В обзоре представлены данные о биохимическом механизме действия o,n'-дихлордифенилдихлоретана (o,n'-ДДД, митотан), который используется для лечения рака коры надпочечников и болезни Кушинга. Под действием препарата возникают многочисленные биохимические изменения в коре надпочечных желез и нарушается структура митохондрий. При этом существенно снижается образование кортикостероидных гормонов. Одной из возможных причин этого может быть снижение уровня NADPH из-за подавления активности системы его восстановления и повышенного расходования для восстановления глутатиона глутатионредуктазой. В надпочечниках o,n'-ДДД частично метаболизируется и основным его метаболитом является o,n'-дихлордифенилнитрозная кислота (с точки зрения количества). Однако попытки найти среди метаболитов физиологически активный компонент были безуспешными. Наиболее выраженные изменения, вызываемые o,n'-ДДД, выявлены в митохондриях коркового вещества надпочечников: подавляется дыхание на уровне
IV и I комплексы, снижается содержание протеинов этих комплексов, изменяется фосфолипидный состав ткани и снижается содержание дифосфатидилглицерола – важнейшего компонента мембран митохондрий. По нашему мнению, о,o′-ДДД из-за высокой липофильности накапливается в мембранах митохондрий и вызывает ее конформационные нарушения с последующими нарушениями функции. Установлено, что о,o′-ДДД является ингибитором ацил-КоА-холестеролацетаттрансферазы (AXAT, SOAT1). Из-за этого в адренокортикоцитах накапливается свободный холестерол, возникает стресс эндоплазматического ретикулума, развивается набухание митохондрий и активируются каспазы. Усиление апоптоза приводит к уменьшению функции железы и снижению ее массы.

Ключевые слова: кора надпочечных желез, стероидогенез, митотан, хлодитан, глутатион, митохондрии, стресс эндоплазматического ретикулума, апоптоз.

References


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