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The adrenal specific toxicant mitotane directly interacts with lipid membranes and alters membrane properties depending on lipid composition

Holger A. Scheidt [a], Ivan Haralampiev [b], Stephan Theisgen [a], Andreas Schirbel [c], Silviu Sbiera [d], Daniel Huster [a], Matthias Kroiss [d], Peter Müller [b, *]

[a] University of Leipzig, Institute of Medical Physics and Biophysics, Härtelstr. 16-18, 04107 Leipzig, Germany
[b] Humboldt University Berlin, Department of Biology, Invalidenstr. 42, 10115 Berlin, Germany
[c] University Hospital Würzburg, Department of Nuclear Medicine, Oberdürrbacher Straße 6, 97080 Würzburg, Germany
[d] University Hospital Würzburg, Department of Internal Medicine I, Endocrinology and Diabetes Unit, Oberdürrbacher Straße 6, 97080 Würzburg, Germany

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ABSTRACT

Mitotane (o,p′.-DDD) is an orphan drug approved for the treatment of adrenocortical carcinoma. The mechanisms, which are responsible for this activity of the drug, are not completely understood. It can be hypothesized that an impact of mitotane is mediated by the interaction with cellular membranes. However, an interaction of mitotane with (lipid) membranes has not yet been investigated in detail. Here, we characterized the interaction of mitotane and its main metabolite o,p′-dichlorodiphenyldichloroacetic acid (o,p′-DDA) with lipid membranes by applying a variety of biophysical approaches of nuclear magnetic resonance, electron spin resonance, and fluorescence spectroscopy. We found that mitotane and o,p′-DDA bind to lipid membranes by inserting into the lipid–water interface of the bilayer. Mitotane but not o,p′-DDA directly causes a disturbance of bilayer structure leading to an increased permeability of the membrane for polar molecules. Mitotane induced alterations of the membrane integrity required the presence of phosphatidylethanolamine and/or cholesterol. Collectively, our data for the first time characterize the impact of mitotane on the lipid membrane structure and dynamics, which may contribute to a better understanding of specific mitotane effects and side effects.

1. Introduction

Mitotane (o,p′-dichlorodiphenyldichloroethane, o,p′-DDD, Lysodren®) is the only drug approved for treatment of adrenocortical carcinoma (ACC) (Hahner and Fassnacht, 2005; Igaz et al., 2008). ACC is an orphan disease with an annual incidence of 0.5–2/million inhabitants (Golden et al., 2009). Complete tumor removal is still the only potentially curative option and is the initial treatment of choice in localized disease (Jurowich et al., 2013; Schteingart et al., 2005). Since local recurrence is frequent, mitotane is recommended by most centers as an adjuvant treatment after complete resection (Terzolo et al., 2007, 2013; De Francia et al., 2012).

In advanced disease, mitotane is a cornerstone of the treatment as well (Fassnacht et al., 2011, 2013; Else et al., 2014), but an objective response to monotherapy is only observed in 20% of the patients (Hahner and Fassnacht, 2005). Importantly, mitotane treatment is complicated by side-effects and drug interactions (Kroiss et al., 2011).

Despite its clinical use for more than five decades and efforts from several groups using various experimental approaches (Moore et al., 1980; Kruger et al., 1984; Jensen et al., 1987; Stigliano et al., 2008; Hescot et al., 2013; Poli et al., 2013) the molecular mechanisms underlying mitotane efficacy in ACC are only poorly understood. Only recently, we provided evidence that mitotane induces endoplasmic reticulum stress specifically in adrenocortical cells by accumulation of toxic lipids. We demonstrated that mitotane directly inhibits the Sterol-O-Acyl Transferase 1 (SOAT1), an enzyme bound to mitochondria-associated endoplasmic reticulum membranes (MAM) of the endoplasmic reticulum, thus explaining a perturbation of lipid homeostasis through mitotane (Sbiera et al., 2015). We also demonstrated that the majority of the cellular

* Corresponding author.
E-mail address: peter.mueller.3@rz.hu-berlin.de (P. Müller).

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Abbreviations

ACC	adrenocortical carcinoma
Chol	cholesterol
o,p′-DDA	o,p′-dichlorodiphenyldichloroacetic acid
HBS	HEPES buffered saline
HBS50	HBS containing 50 mM HEPES
DOPC	1,2-dioeloyl-*sn*-glycero-3-phosphocholine
DOPE	1,2-dioeloyl-*sn*-glycero-3-phosphoethanolamine
DOPS	1,2-dioeloyl-*sn*-glycero-3-phosphoserine
LUVs	large unilamellar vesicles
Mitotane	o,p′-dichlorodiphenyldichloroethane
NBD-PC	1-palmitoyl-2-(12-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine
NBD-PE	1-palmitoyl-2-(12-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphoethanolamine
NBD-PS	1-palmitoyl-2-(12-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphoserine
POPC	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
POPC-d_{31}	perdeuterated POPC
POPE	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine
POPE-d_{31}	perdeuterated POPE
PS	phosphatidylserine
SL-Chol	25-doxyl-cholesterol
SL-PC	1-palmitoyl-2-(4-doxylpentanoyl)-*sn*-glycero-3-phosphocholine
SL-PE	1-palmitoyl-2-(4-doxylpentanoyl)-*sn*-glycero-3-phosphoethanolamine
SL-PS	1-palmitoyl-2-(4-doxylpentanoyl)-*sn*-glycero-3-phosphoserine.

uptake of a radioactive mitotane analog occurred in association with lipids (Sbiera et al., 2015). Along these lines, recently the majority of mitotane circulating in the blood was found to be bound to lipoproteins (Kroiss et al., 2016; Hescot et al., 2015). Notably, a main metabolite of mitotane, o,p′-dichlorodiphenyldichloroacetic acid (o,p′-DDA), is inactive *in vitro* regarding induction of the hormonal and cytotoxic effects observed with mitotane (Hescot et al., 2014) although in a clinical setting, determination of o,p′-DDA in serum may provide additional information about tumor response (Hermsen et al., 2011).

From a physicochemical point of view, mitotane and o,p′-DDA (Fig. 1) are very likely to interact with lipid membranes given the lipophilicity of their aromatic ring structures. For similar molecules like local anesthetics and other aromatic compounds a localization in the lipid–water interface of the membrane has been found by various biophysical methods (Yau et al., 1998; Huster et al., 2001; Scheidt et al., 2004; Weizenmann et al., 2012). In these cases, membrane interaction was shown to be caused by the hydrophobic effect, favorable dipole–dipole interactions, and hydrogen bonding to more polar lipid groups (Yau et al., 1998; Huster et al., 2001).

To the best of our knowledge, only a single publication has dealt with the impact of mitotane on biological membranes (Jacobi et al., 2014). By using erythrocyte cell membranes after mitotane exposure, intracellular Ca^{2+} was found to be increased, which caused the appearance of phosphatidylserine (PS), a lipid which is normally localized on the inner membrane leaflet (Zachowski, 1993), on the outer membrane leaflet. Intriguingly, enhanced transbilayer phospholipid movement leading to the accumulation of PS in the outer membrane leaflet is a feature of apoptosis (Bevers et al., 1999; Bevers and Williamson, 2010), a process triggered by mitotane in ACC tumor cells (Lehmann et al., 2013; Sbiera et al., 2015). The mechanism(s), by which mitotane affected the erythrocyte membrane, were not explored further.

The current study aims at characterizing the interaction of mitotane and o,p′-DDA with lipid bilayers focusing on the two most important constituents of biological membranes, phospholipids and cholesterol. In particular, the interaction of mitotane and o,p′-DDA with lipid vesicles of defined composition was investigated,

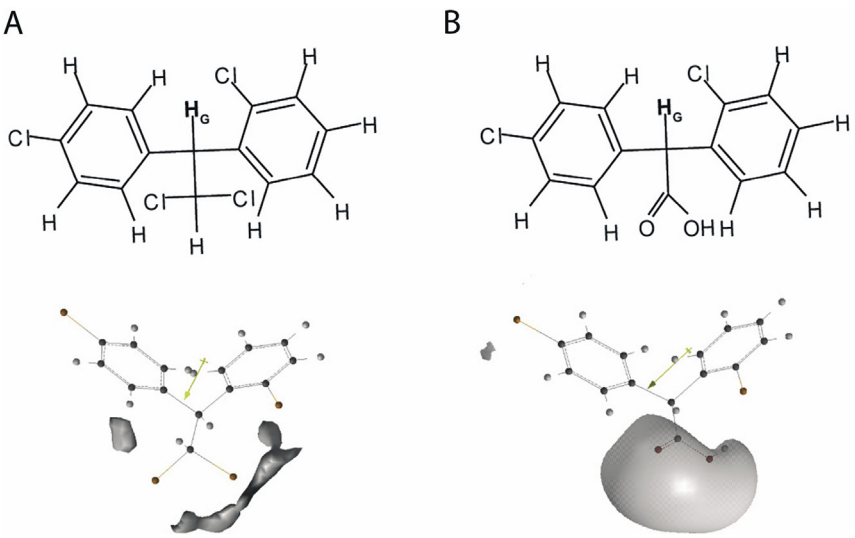

Fig. 1. Chemical structures and isosurfaces of the electrical potential of mitotane (A) and o,p′-DDA (B). The molecular dipole moment is indicated by the arrows in the isosurface plots.

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