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Exploring mitochondrial cholesterol (mChol) signalling for therapeutic intervention in neurological conditions

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Abstract

The pharmacological targeting of cholesterol levels continues to draw interest due to the vast success of therapeutics such as statins in extending life expectancy by modifying the prognosis of diseases associated with the impairment of the lipid metabolism. Advances in our understanding of mitochondrial dysfunction in chronic age-related diseases of the brain have unveiled an emerging role for mitochondrial cholesterol (mChol) in their pathophysiology, thus delineating an opportunity to provide mechanistic insights and explore strategies of intervention. This review draws attention to novel signalling mechanisms in conditions linked with impaired metabolism associated with impaired handling of cholesterol and its oxided forms (oxysterols) by mitochondria. By emphasising the role of mChol in neurological diseases we here call for novel approaches as well as new means of assessment.

Keywords: Mitochondria, Neurodegeneration, cholesterol, oxysterol,

Abbreviations

mChol	Mitochondrial cholesterol
ER-MAM	endoplasmic reticulum-mitochondria associated membrane
ER	endoplasmic reticulum
mtDNA	mitochondrial DNA
AD	Alzheimers disease
ROS	reactive oxygen species
7β-OHC	7β-hydroxycholesterol
7-KC	7-ketocholesterol
CNS	central nervous system
IMM	Inner mitochondrial membrane
ATAD3	ATPase family AAA domain-containing protein 3
APOE4	apolipoprotein isoform 4
LDLr	low density lipoprotein receptor
AßPP	amyloid ß precursor protein
Aß	amyloid ß peptide
StAR	steroidogenic acute regulatory protein
VDAC1	voltage-dependent anion selective channel 1
ACBD1/3	Acyl-coenzyme A binding domain containing 3
TSPO	translocator protein
OMM	Outer Mitochondrial Membrane
ANT	adenine nucleotide transporter
CYP11A1	Cytochrome P450 Family 11 Subfamily A Member 1
PD	Parkinson's Disease
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP^+	1-methyl-4-phenylpyridinium
HD	Huntington's Disease
HTT	huntingtin protein
ALS	amyotrophic lateral sclerosis
24-OHC	24-hydroxycholesterol
27-OHC	27-hydroxycholesterol
4α-OHC	4α-hydroxycholesterol
4β-OHC	4β-hydroxycholesterol
α-epoxy C	α-epoxy cholesterol
β-epoxy C	β-epoxy cholesterol
25-OHC	25-hydroxycholesterol
7α-OHC	7α-hydroxycholesterol
SOD1	superoxide dismutase 1
LXR	liver-X receptor
MitoQ	10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl triphenylphosphonium methane sulfonate
CoQ10	Coenzyme Q10
SkQ1	10-(6'-plastoquinonyl) decyltriphenylphosphonium

Introduction

Cholesterol has emerged as a keystone lipid in mammalian cellular physiology and pathology since it was identified as bile solid in gallstones in 1769 by François Poulletier de la Salle. Cholesterol promotes an increase in lipid conformational order thus providing protection to animal cells (Simons & Ikonen, 1997) besides being a precursor for steroid hormones, bile acids and vitamin D. The insertion of cholesterol into various organellar membranes lends rigidity to these and offers protein-tethering platforms such as in the case of synaptic lipid rafts or ER-MAM (Endoplasmic Reticulum-mitochondria associated membrane) structures (Fujimoto, Hayashi & Su, 2012; Lajoie, Goetz, Dennis & Nabi, 2009).

Cholesterol is loaded differently among organelles and intracellular compartments. For instance, the plasma membrane contains cholesterol 40-fold higher than the ER and mitochondria (Horvath & Daum, 2013). In the mitochondria cholesterol is a: (i) structural component of the inner and outer mitochondrial membranes; (ii) precursor of steroidogenesis (of which the first steps are conducted in the mitochondrial lumen); (iii) core to a platform of interaction with ER, lysosomes and other compartments; as well as (iv) a tethering element for mitochondrial DNA (mtDNA). As a consequence, alterations in mChol occur in several diseases amongst which Alzheimer's disease (AD) and neurodegenerations (Desai et al., 2017; Elustondo, Martin & Karten, 2017). The juxtaposition of mChol with the respiratory chain complex where reactive oxygen species (ROS) are produced, creates ideal conditions for the production of auto-oxidative products of cholesterols: the oxysterols (Zerbinati & Iuliano, 2017) which are implicated in brain diseases too. Some oxysterols are intermediates of cholesterol metabolism, enzymatically transformed into bile acids, steroid hormones and vitamin D. Auto-oxidation of cholesterol by ROS also results in the formation of oxysterols implying a pro-pathological positive feedback which amplifies mitochondrial dysfunction and hence severity of the condition.

7β-hydroxycholesterol (7β-OHC), 7-ketocholesterol (7-KC) and 5,6-epoxides as well as the secosterols are all produced under oxidative stress. In keeping with this, the levels of several species of oxysterols reflect the degree of pathology in chronic Central Nervous System (CNS) conditions (Zerbinati & Iuliano, 2017). It remains unclear though, how these processes can be pharmacologically modulated to inform therapeutic protocols or re-purposing existing cholesterol targeting chemicals. Here

we overview our current knowledge of mChol homeostasis and its link to neurodegeneration to stem interest and encourage further exploitation of this lipid in mitochondrial physiopathology.

Role of cholesterol in Mitochondrial DNA maintenance

mtDNA is associated with nucleic acid binding proteins forming complexes known as nucleoids (Spelbrink, 2010). Mutations in mtDNA as well as in nuclear-encoded mitochondrial genes cause primary mitochondrial diseases (Gorman et al., 2016). Cholesterol rich patches in the mitochondrial inner membrane tether the mtDNA to the inner mitochondrial membrane (IMM) via nucleoprotein complexes called nucleoids. These patches and their components enable mtDNA processing, protein synthesis and replication (Gerhold et al., 2015; He et al., 2007). While the lipid composition of mitochondria has been described (Fleischer, Rouser, Fleischer, Casu & Kritchevsky, 1967), dynamics of distribution and regulation remain fairly unexplored. Mitochondria are 'cholesterol-poor' organelles with a cholesterol to phospholipid ratio as low as 0.1 (van Meer, Voelker & Feigenson, 2008). This low level of cholesterol is unlikely to form classical lipid rafts with close association with sphingolipids (Zheng, Berg & Foster, 2009). However, from what is known about the behaviour of bilayer membranes and lipid movement, it can be inferred that cholesterol is restricted to nanodomains in the strict curvatures of the IMM (Rukmini, Rawat, Biswas & Chattopadhyay, 2001). This implies that rather than cholesterol-poor mitochondria they are cholesterol-vital organelles. The majority of primary mitochondrial diseases caused by mutations in the mtDNA associates with neurological deficits, ranging from mild ataxia to severe early onset of neurodegeneration (Carelli & La Morgia, 2018). Explorations into the mChol modulating proteins suggest that they directly impact mtDNA and hence associated mutations cause severe primary mitochondrial disorders.

One of the components of nucleoprotein complex associated with mtDNA is the AAA+ ATPase protein ATPase family AAA domain-containing protein 3 (ATAD3), which has mtDNA binding properties (He et al., 2012; He et al., 2007). ATAD3 was found to affect the rate of steroidogenesis by facilitating cholesterol transport from ER to mitochondria (He et al., 2012; He et al., 2007; Issop et al., 2015). Mutations in the ATAD3 family of proteins which alter cholesterol metabolism, cause severe neurodegeneration, mitochondrial cristae defects and impaired mtDNA segregation (Desai et al., 2017;

Peralta et al., 2018). Pharmacologically interfering with cholesterol shuttling in ATAD3 deficient human fibroblasts via U18666A or by altering cholesterol biosynthesis via statins (e.g. pravastatin), results in exacerbated mtDNA de-segregation. Aggregation and disorganization imbalance are also observed in the Niemann-Pick type C disorder, further supporting the critical role of cholesterol inserts in mitochondria by controlling the tuned segregation of the organelle DNA (Desai et al., 2017).

More recently, it has been also shown that deficiency in ATAD3 affects the formation of mitochondrial cristae (Peralta et al., 2018) suggesting that the optimum level of cholesterol inserts into the IMM is equally crucial in maintaining membrane structure as well as mtDNA integrity. Dysregulation of mChol may therefore result in primary mitochondrial dysfunction perturbing mtDNA homeostasis leading to deficits in the energy balance (**Figure 1**).

Mitochondrial cholesterol and oxysterols in neurodegeneration

Several studies have reported dysregulated cholesterol metabolism in AD and the E4 variant of cholesterol gene *apolipoprotein E*(APOE) is a common risk factor for familiar AD [as comprehensively reviewed in (Arenas, Garcia-Ruiz & Fernandez-Checa, 2017)]. However, less is understood about mChol in the disease. In a model of hypercholesterolemia, where low density lipoprotein receptor ($LDLr^{-/-}$) mice are fed a high cholesterol diet, the mice develop cholesterol loading in the mitochondria and subsequent cognitive deficiencies and AD mimicking neurodegeneration. The cerebral cortex of $LDLr^{-/-}$ mice fed with cholesterol-enriched diet showed a (i) decrease in the activities of mitochondrial complexes I and II (ii), glutathione levels (iii), imbalance between the peroxide-removing-related enzymes (glutathione peroxidase and glutathione reductase) (de Oliveira et al., 2011).

Del Prete et al studied a mutant form of Amyloid-β precursor protein (A-βPP) and found that there was an increased incidence of ER-MAM structures, which in turn captured more of the secretase-processed metabolites of the mutant A-βPP in this micro-region thus interfering with MAM functions (Del Prete et al., 2017).

This adds to the growing evidence that ER-MAM interactions are key platforms of AD aetiology (Area-Gomez et al., 2018). The AD peptide Amyloid-ß (Aß), when targeted to mitochondria, is thought to be crucially involved in associated toxicity. Aß induces

ER stress leading to the increased synthesis of cholesterol and loading into the mitochondria via ER-MAM structures. Additionally, enrichment of cholesterol in mitochondrial membranes is reported in AD pathology.

Mitochondria from a mouse model of cholesterol overload exhibit-increased susceptibility to Aß-induced oxidative stress and consequent cytochrome c release (Fernandez, Llacuna, Fernandez-Checa & Colell, 2009). Coupled with this observation, loading of mitochondrial cholesterol is increased in AD mouse model (Fernandez, Llacuna, Fernandez-Checa & Colell, 2009), accompanied by an overexpression of the Steroidogenic Acute Regulatory (StAR) protein (Barbero-Camps, Fernandez, Baulies, Martinez, Fernandez-Checa & Colell, 2014; Hashimoto et al., 2018). StAR is a lipo-protein that transports cholesterol from the ER to mitochondria regulating the intra-organelle distribution of the lipid.

Components of the “transduceome” are known to affect cholesterol processing in the mitochondria and steroidogenesis -including neurosteroidogenesis- making them an attractive target for pharmacological regulation of these important biological processes (Rone, Fan & Papadopoulos, 2009; Rone et al., 2012; Strobbe & Campanella, 2018). The transduceome, which has been studied more extensively in non-neuronal cells, is a complex of cholesterol binding proteins that orchestrate movement of cholesterol into the mitochondria. It comprises, the Voltage Dependent Anion Channel 1 (VDAC1) along with interacting partners, ACBD1/3, and, under steroidogenic conditions, Translocator Protein (TSPO) and StAR on the outer mitochondrial membrane (OMM). They connect with ATAD3 on the IMM via adenine nucleotide translocase (ANT) to deliver cholesterol to the processing enzyme Cytochrome P450 Family 11 Subfamily A Member 1 (CYP11A1) to generate pregnenolone - the first step of steroidogenesis (Rone, Fan & Papadopoulos, 2009; Rone et al., 2012). Under conditions of stress, such as neuroinflammation and neurodegeneration, the 18kDa TSPO is over-expressed to fuel the cholesterol processing machinery (**Figure 1**). Although the precise function of TSPO remains unresolved and likely boarder (Gatliff et al., 2014), the protein presents two validated cholesterol binding sites (Fantini, Di Scala, Evans, Williamson & Barrantes, 2016; Jaipuria et al., 2017; Jaremko, Jaremko, Giller, Becker & Zweckstetter, 2014). When bound to cholesterol TSPO changes confirmation (Jaipuria et al., 2017) implying that it has a key function in regulating intra-organellar distribution of cholesterol. As a result of a druggable structure (Jaremko, Jaremko,

Giller, Becker & Zweckstetter, 2014) and temporally regulated expression, (Gavish & Veenman, 2018) TSPO has been a target of several generations of chemical PET tracers (positron emission tomography) and pharmaceutical ligands (Veenman, Vainshtein, Yasin, Azrad & Gavish, 2016) (see **Table 1**).

In our opinion this represents an opportunity for exploration of potential protective effects by disrupting the transduceome with TSPO regulators (Gatliff & Campanella, 2016). A better understanding of TSPO function, and its role of the steroidogenic transduceome in neuronal dysfunction is therefore necessary to lay a foundation for foreseeable therapeutic interventions. While cholesterol synthesis inhibitors such as the "blockbuster" statins (Cholesterol Treatment Trialists et al., 2015) occupy an elite place in cholesterol modulation, it is perhaps time to look beyond this strategy and turn to more subtle and selective mechanisms of cholesterol shuttling. The TSPO ligands are indeed exploited for anti-inflammatory and neuroprotective effects (Qiu et al., 2016; Scholz et al., 2015).

In a rat model of hypercholesterolaemia, ischemia-reperfusion injury results in mitochondrial sterol (both cholesterol and oxysterol) accumulation in mitochondria (Paradis, Leoni, Caccia, Berdeaux & Morin, 2013), which can be ablated by TSPO ligands SSR180575 (benzodiazepine), 4′-chlorodiazepam or TRO40303. This methodology may prove useful in diseases of the CNS where mitochondrial sterol levels are altered and the range of TSPO ligands (annotated in **Table 1**) can be a useful toolkit to explore this.

Within the context of mitochondrial dysfunction, classically, the research on Parkinson's disease (PD) has focussed on the deficiencies in quality control regulation of mitochondria by autophagy (Larsen, Hanss & Kruger, 2018). However, studies have shown that disrupted cholesterol dynamics associate with established molecular features of PD [reviewed in (Arenas, Garcia-Ruiz & Fernandez-Checa, 2017)]. One of the earliest evidence was in PD patients derived human fibroblasts which showed 50% reduction in cholesterol biosynthesis (Musanti, Parati, Lamperti & Ghiselli, 1993).

Lim et al found that a cholesterol precursor lanosterol was 50% lower in a neurotoxin-induced mouse model (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) of PD. This evidence was recapitulated *in vitro* where it was observed a redistribution of lanosterol synthase from the ER to mitochondria in dopaminergic neurons exposed to 1-methyl-4-phenylpyridinium (MPP^+) thus implying a survival effect via mitochondria (Lim et al., 2012). Similarly, in neuroblastoma cells treated with MPP^+ there was

marked accumulation of cholesterol in lysosomes (Eriksson, Nath, Bornefall, Giraldo & Ollinger, 2017). When this effect was mimicked by cholesterol blocking agent U18666A, cell death was reduced, hinting that lysosomal cholesterol accumulation may be an adaptive stress response. Furthermore, cholesterol synthesis inhibitor lovastatin reduced MPP^+- induced cell death by lowering ROS production without preventing the accumulation of cholesterol into lysosomes (Eriksson, Nath, Bornefall, Giraldo & Ollinger, 2017). This argues for further functions for mtChol: alike the ER-MAM communication also the lysosome-mitochondria communication is cholesterol dependent and the delicate balance of membrane lipid composition critical for healthy CNS.

A recent publication by Lin et al (Lin et al., 2018), has shone light on the transcriptomic changes accompanying the isoform change of Apolipoprotein (APOE3 → APOE4) which has for long been the largest genetic risk factor acknowledged for late onset sporadic AD. The authors dissected the transcriptomic changes in different types of derived CNS cells discovering that astrocytes, neurones, and microglia regulate different pathways to compensate for the loss of APOE4. Most notably, APOE4 astrocytes have altered cholesterol metabolism, this is of particular interest because astrocytes are known to supply cholesterol to neurons. The gene-edited CNS cells exhibit other features of AD such as compromised A-β clearance, altered synaptic formation, immune activation, increased A-β production and hyperphosphorylated tau. Lin et al present in dish modelling of AD and a scientific validation for exploring cholesterol shuttling as a target for therapy. Perhaps, this balance of cholesterol at interorganellar interactions is key to mitochondrial network dynamics that ultimately define cellular health, especially in neurones, which are highly dependent on oxidative phosphorylation. In the case of familiar PD, in which deficiency impacts mitochondrial quality control by autophagy to the extent of dysfunction and ultimately death of the dopaminergic neurones, the ability to modulate mitophagy can prove critical. Mitochondrial cholesterol protein TSPO has an anti-mitophagy effect when overexpressed (Gatliff & Campanella, 2015; Gatliff et al., 2014).

By disrupting the activity of TSPO via its ligands or changing its residence time on the mitochondria, efficient mitophagy could be restored and so cellular health. Intriguingly, modulation of mChol may allow the same beneficial outcome. This is supported by observations in pre-clinical as well as clinical studies, in which TSPO expression

correlates with longitudinal progression of neurological conditions (Cumming & Borghammer, 2012; Maia et al., 2012).

Unlike AD, other neurodegenerative diseases do not have such straightforward evidence of mChol involvement in their pathophysiology. We nonetheless know that Huntington's disease (HD), the HTT (huntingtin protein)-induced mitochondrial fluidity can be rescued by olesoxime, a cholesterol-like product (Eckmann et al., 2014) which bears also neuroprotective effects in amyotrophic lateral sclerosis (ALS) (Martin, 2010).

Oxysterols – Schrodinger's cat of mitochondrial cholesterol related dysfunction

Cholesterol is present at the site of mitochondrial ROS production and susceptible to auto-oxidation into oxysterols making of these a metaphorical Schrodinger's cat of neurodegeneration. Heightened oxysterol levels are being used as biomarkers for neurodegenerative diseases and lysosomal storage disorder progression (Griffiths et al., 2017; Testa et al., 2016). Cholesterol can be enzymatically broken down into 24-hydroxycholesterol (24-OHC) and 27-hydroxycholesterol (27-OHC), or auto-oxidised by ROS to products like 7-KC, 4α-hydroxycholesterol (4α-OHC), 4β-hydroxycholesterol (4β-OHC), 7β -OHC, α-epoxy cholesterol (α-epoxy C) and β-epoxy cholesterol (β-epoxy C), while 25-hydroxycholesterol (25-OHC) and 7α- hydroxycholesterol (7α-OHC) can occur in both ways. When tested in AD (Alzheimer's disease) patients, all of the above tested oxysterols are found elevated in late stage of the condition except for the enzymatic 24-OHC, which reduces with disease progression (Testa et al., 2016).

ALS is a primary target of exploited oxysterol signalling. Since the discovery that mutation in the mitochondrial anti-oxidant enzyme superoxide dismutase 1 (SOD1) can cause ALS (Ince, Shaw, Slade, Jones & Hudgson, 1996), mitochondrial oxidative stress in motor neurones has become a key research interest in the field. Recent studies have revealed that liver x receptors α and β (LXRα and LXR-β) are key players in ALS aetiology. LXRs are nuclear receptors of oxysterols, which regulate cholesterol synthesis amongst other cellular processes (dependent on the cellular type). A recent study identified two single nucleotide polymorphisms of LXRα, rs2279238 and

rs7120118 associated with delayed age of ALS onset amongst a cohort of 330 ALS patients (Mouzat, Raoul, Polge, Kantar, Camu & Lumbroso, 2016). Moreover, male LXR-$\beta^{-/-}$ mice develop severe motor impairment closely resembling ALS at 7 months which later progresses to hind-limb paralysis (Andersson, Gustafsson, Warner & Gustafsson, 2005).

If autoxidative forms of oxysterols produced in the mitochondria are acknowledged contributing factors in ALS, beneficial are the antioxidant protocols adopted to ameliorate the condition. Miquel et al assessed the therapeutic benefit of a mitochondrially targeted anti-oxidant MitoQ (10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl) decyl triphenylphosphonium methane sulfonate) in a mouse model of familial ALS in which decline of mitochondrial function was slowed down, in both the spinal cord and the quadriceps muscle (Miquel et al., 2014).

While the preclinical data are substantive, no anti-oxidant therapy has, hitherto, proved to modify ALS or any other neurodegenerative condition. Intriguingly though, there are some reports of long term statins usage causing ALS, which is attributed to the parallel reduction of mitochondrial antioxidant Coenzyme Q10 (CoQ_{10}) by the inhibition of HMG-CoA reductase enzyme (Edwards, Star & Kiuru, 2007).

Testing other anti-oxidants such as 10-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1), MitoQuercetin, Mitocurcumin, Mitoresveratrol, MitoHonokiol, Mitoapocynin, AntiOxCIN4, AntiOxBEN2, could anyway prove beneficial in ALS, more importantly in the models where the anti-oxidant system is disrupted (Teixeira, Deus, Borges & Oliveira, 2018).

After almost 2 decades since the approval of Riluzole for ALS, only recently Edaravone was approved by the FDA (May 2017), showing benefit in a randomized double-blind clinical trial (Rothstein, 2017; Writing & Edaravone, 2017). While Edaravone has other effects, such as reducing inflammation, its main activity is anti-oxidant. While it is not mitochondrially targeted, there is a possibility that by quenching ROS it facilitates the modulation of oxysterols and mChol for therapeutic benefit. This strategy has not been tested in neurological conditions but warrants further investigation on this.

The interest in LXRs as therapeutic targets has steadily increased for a multitude of diseases ranging from vascular to metabolic and the neurological ones. Potent and selective LXR ligands continue to emerge from screening of small molecule libraries, rational design and empirical medicinal chemistry approaches. In spite of this,

challenges remain in minimizing undesirable effects of LXR activation on lipid metabolism (Komati, Spadoni, Zheng, Sridhar, Riley & Wang, 2017) for which a mitochondrial health assay approach may prove useful to implement drug screening (**Figure 2**).

Conclusions and Perspectives

All these evidences indicate that mChol has been well studied in the context of steroidogenesis but largely ignored in neurodegeneration. While there are the known functions of (i) precursor to steroids (ii) ER-MAM and (iii) Mitochondrial-Lysosomal interaction, as well as (iv) mtDNA tethering, there are other biochemical or mechanobiological processes that involve homeostasis of the cholesterol in the mitochondria which remain il-defined.

With the revelation that ER-MAMs are important in AD (i), the mitochondria-lysosomal interaction is compromised in PD (ii), and disruption of oxysterol signalling can lead to ALS (iii), mChol stands as a logical target to inform and treat these conditions. Furthermore, a dissection of oxysterol signalling could per se lead to identification of potential therapeutic avenues in neurodegeneration. The genetic evidence in ALS advocate for this to be the case as equally strong are those from studies with SOD1 mutants as well as LXR-$\beta^{-/-}$ mice: lack of ROS neutralization as well as dysregulated oxysterol signalling lead to motor neuron degeneration (Abdel-Khalik et al., 2017; Mouzat et al., 2018; Mouzat, Raoul, Polge, Kantar, Camu & Lumbroso, 2016). Most notably, increasing anti-oxidant levels improve the tone of cholesterol signalling via LXRβ leading to a beneficial outcome in neurodegeneration (Bond, Bernhardt, Madria, Sorrentino, Scelsi & Mitchell, 2018; Sandoval-Hernandez, Restrepo, Cardona-Gomez & Arboleda, 2016; Stachel et al., 2016). Along with the need to continue gathering evidences on the beneficial effect of cholesterol modulating agents on neurodegeneration, it is therefore pivotal assessing novel means of measure of neuroprotection such as handling of the lipid by mitochondria.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the

IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

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Competing Interests' Statement

R.D. and M.C declare no Financial competing interests with the matter of this publication.

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Figure 1. The pivotal role of cholesterol in cellular physiology and pathology
Cholesterol (green) serves multiple purposes in the mitochondria (i) As a structural component of the inner and outer mitochondrial membrane (OMM, IMM) (ii) As a precursor of steroidogenesis, of which the first steps are conducted in the mitochondrial lumen (iii) As providing the platform for interorganellar interaction with endoplasmic reticulum (ER), Lysosomes (LY) and other intracellular compartments, and (iv) as the tether for mitochondrial DNA (mtDNA).

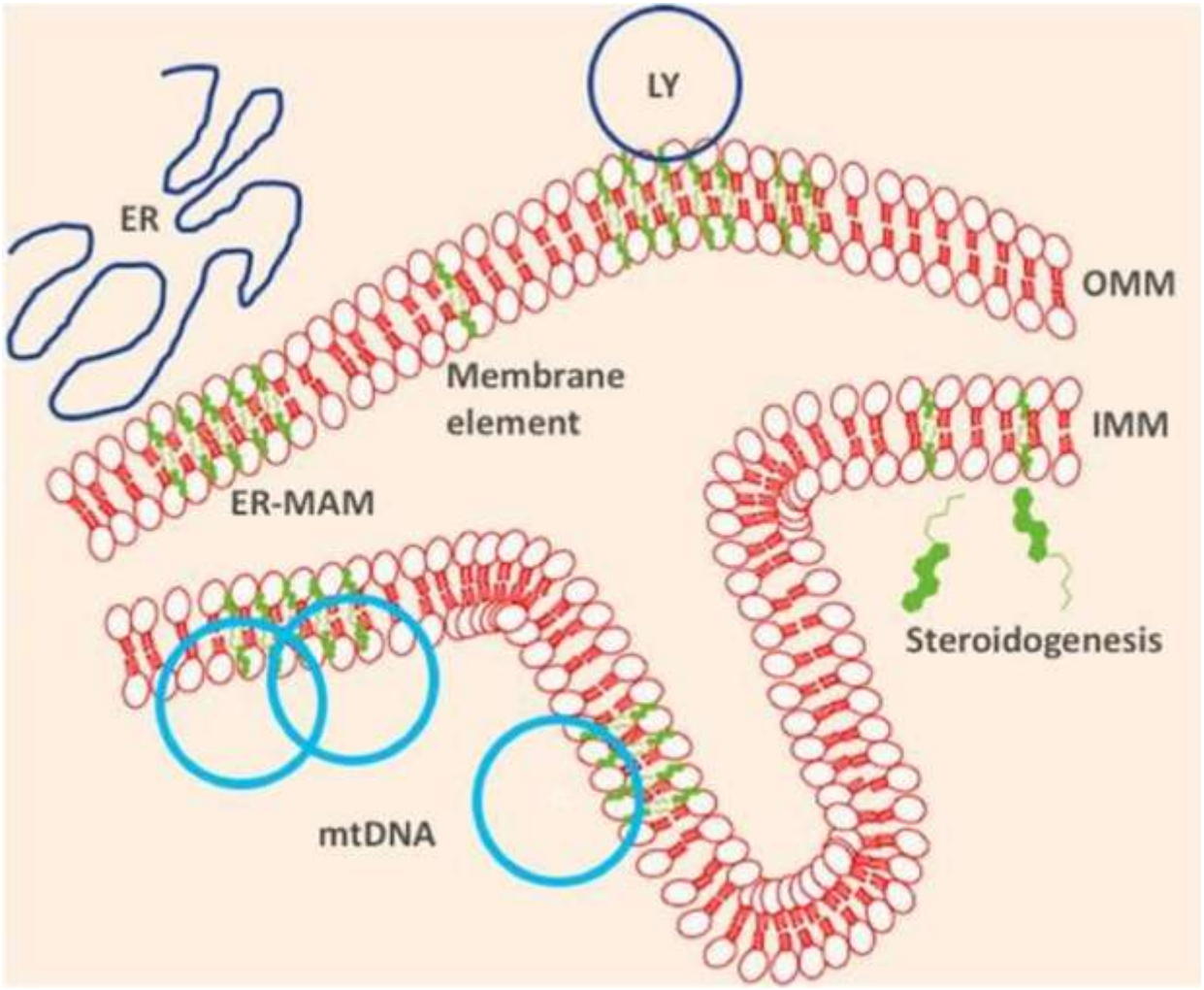
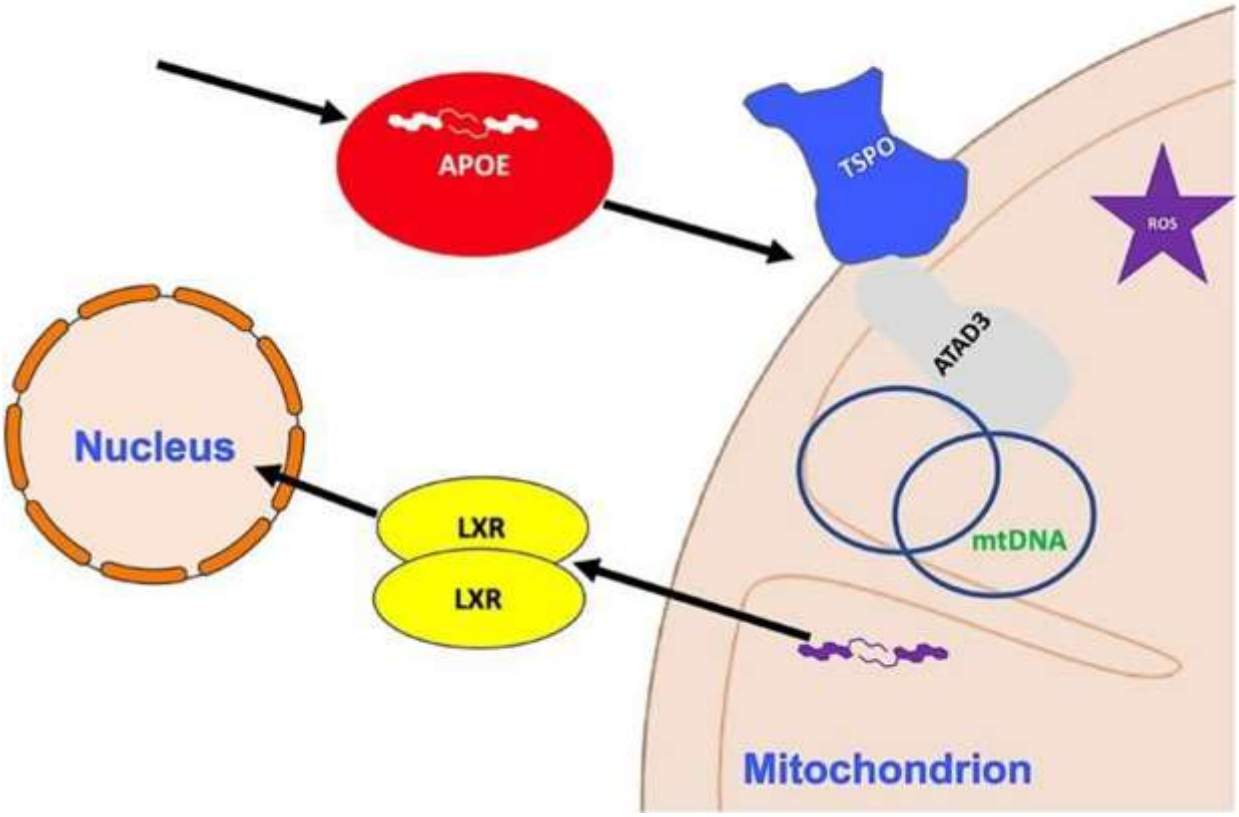
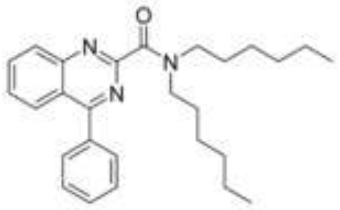
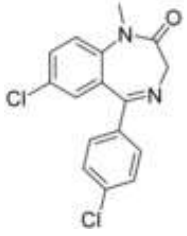


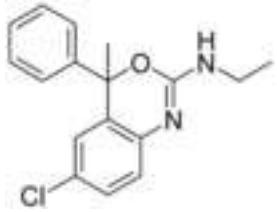
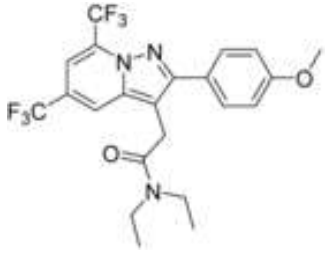
Figure 2. Cholesterol interplay between mitochondria and cytosol

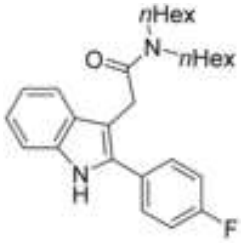
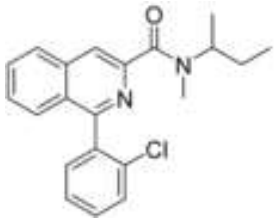
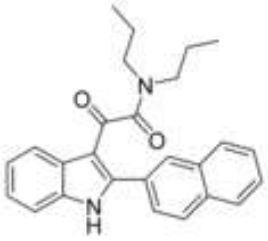
The picture depicts the prominent mechanisms of cholesterol import (e.g. TSPO) in the mitochondria exploited by the intracellular accumulation of APOE. The two isoforms of the transcription factor liver X receptor (LXRα and LXRβ) activated and hence translocated in the nucleus by the oxidized derivatives of cholesterol (oxysterols), which are formed by the high redox stress produced by malfunctioning mitochondria are also highlighted in the scheme.

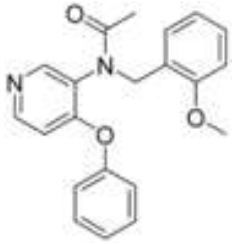
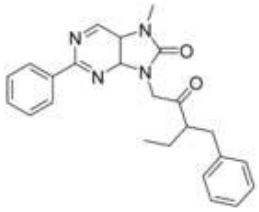
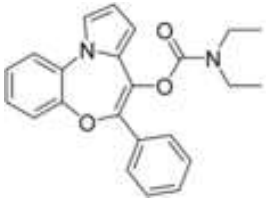
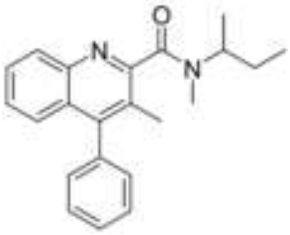


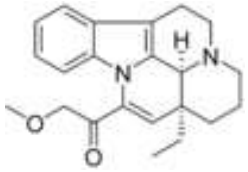
The table lists the ligands of the Translocator Protein (TSPO), their chemical structure, pharmacological effect, clinical or pre-clinical use.

Class	Compound	Structure	Properties
4-Phenylquinazoline-2-carboxamides	ER176		Aza-isosteres of PK11195. In particular, PET radioligands with sensitivity to robustly image all three TSPO genotypes in human brain
Benzodiazepines	Ro-5-4864		Sedative, neuroprotective, Agonist or partial agonist of TSPO with nanomolar binding affinity

Benzoxazines	Etifoxine		Anxiolytic effects, anti- neurodegenerative effects mediated by TSPO, PET ligand
Cholest-4-en-3-one	TRO40303		Agonist, used in cardioprotection, ALS, Putatively interrupts the formation of mitochondrial transition pore
Imidazopyridineacetamides	DPA		Ligand used for in vivo imaging of TSPO

Indoleacetamides	FGIN-1-27		TSPO ligand characterized by steroidogenic and pro-apoptotic activities
Isoquinoline carboxamides	PK11195		TSPO antagonist with nanomolar affinity, widely used for characterizing expression and function in various tissues and cells, Widely used PET ligand
N,N-Dialkyl-2-phenylindol-3-ylglyoxylamide (PIGA)	PIGA 1128		Used to modify steroidogenic activity of TSPO, specifically in relation to neurosteroids. Developed for anxiolytic activity.

Phenoxyphenylacetamides	PBR28		Developed as PET ligands for TSPO, specifically used for neuroinflammation. Brain penetrant.
Phenylpurines	Emapunil		Ligand with rapid anxiolytic effects
Pyrrolobenzoxazepines	OXA-17		Developed as anti-cancer therapies, some activity for cannabinoid receptors.
Quinoline carboxamides	VCM198M		Used as radioligand for TSPO imaging

Vinca alkaloids	Vinpocetine		Ligand with neuroprotective activity that binds TSPO and other receptors such as adrenergic receptors