

## Drug Induced Mitochondrial Toxicity: Mechanistic Diversity and Deleterious Consequences for the liver

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### ABSTRACT

Drug-induced liver injury (DILI) has become a leading cause of severe liver disease and withdrawal of an approved drug from the market. DILI accounts for acute liver failure, liver transplantation or death in the United States today. A recent retrospective study indicates that the risk of DILI is enhanced when the administered daily dosage is higher than 50 mg or when the drug undergoes significant liver metabolism. Hence it poses a major clinical and regulatory challenge.

This review summarizes direct mitochondrial impairment and specific drug-induced mitochondrial dysfunction, current mechanistic concepts of DILI in a 2-step model that limits its principal mechanisms to these main ways of initial injury. Numerous studies that evaluate the risk of hepatotoxicity from statins in hyperlipidemic patients. In this article, it will review the pathogenesis of drug-induced mitochondrial liver toxicity and deleterious consequences of atorvastatin.

**Keywords:** Hepatic, Idiosyncratic, Mitochondrial, Hyperlipidemic, Statins, Drug-induced liver injury (DILI)

### INTRODUCTION

More than 1000 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.<sup>[1]</sup> The mechanisms of DILI are not always known, but when they are investigated mitochondrial dysfunction is often present. Importantly, drug-induced mitochondrial dysfunction can be due to the drug itself and/or to reactive metabolites generated through cytochrome P450-mediated metabolism. These mitochondrial disturbances can have a variety of deleterious consequences, such as oxidative stress, energy shortage, accumulation of triglycerides (steatosis), and cell death.<sup>[2]</sup>

### MITOCHONDRIAL STRUCTURE & FUNCTIONS

Mitochondrial membrane permeabilization and cell death:

Mitochondria are organelles with two membranes surrounding a space (matrix) containing various enzymes and the mitochondrial genome (mtDNA). The inner membrane, which also harbors many enzymes, behaves as a barrier that is poorly permeable to various molecules<sup>[3]</sup>. Thus, this membrane contains transporters allowing the entry of endogenous compounds (ADP, fatty acids, glutathione, pyruvic acid) and possibly xenobiotics as well. In some pathophysiological circumstances, the mitochondrial membranes can lose their structural and functional integrity, in particular after the opening of the mitochondrial permeability transition pores (MPTP)<sup>[4]</sup>. These pores involve at least 4

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candidate proteins, namely the peripheral benzodiazepine receptor (PBR), the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase (ANT), and Cyclophilin D<sup>[4]</sup>. The later protein (a modulator of the pore rather than a MPTP component per se<sup>11</sup>) is able to bind the immunosuppressive drug cyclosporine A that therefore reduces the opening probability of the MPTP. In contrast, several drugs and toxic compounds, but also high levels of some endogenous derivatives (e.g. calcium, fatty acids, and bile salts) can induce MPTP opening. As the latter event strongly alters mitochondrial function and structure, it can endanger cell life. However, the exact pathway whereby the cell will die (namely apoptosis or necrosis) depends on the number of mitochondria harboring opened MPTP<sup>[5-7]</sup>. If numerous mitochondria present opened MPTP, ATP stores will slump rapidly and necrosis will occur through a sudden rise in intracellular calcium levels because ATP is mandatory for the activity of the plasma membrane calcium ATPase (PMCA), an enzyme responsible for calcium extrusion out of the cell. In contrast, if MPTP opening takes place only in some mitochondria, ATP levels will be maintained thanks to undamaged organelles. However, the rare mitochondria involved in MPTP opening will swell allowing the release of different pro-apoptotic proteins including the apoptosis inducing factor (AIF), several caspases, and cytochrome C<sup>[8]</sup>. This key protein of the respiratory chain, when released in the cytoplasm, can bind to the Apaf-1 protein and ATP thus initiating the apoptotic pathway through the activation of caspases 9 and 3. Consequently, MPTP opening in a few mitochondria can also have deleterious consequences.

Several important points must be discussed regarding mitochondrial membrane permeabilization. Firstly, MPTP opening initially permeabilizes the mitochondrial inner

membrane without alteration of the outer membrane. However, MPTP opening causes an equilibration of solutes with molecular masses up to 1500 Da and the massive entry of water into the matrix, which causes unfolding of the inner membrane and mitochondrial swelling. The latter event thus induces outer membrane rupture and the release of several mitochondrial proteins located in the intermembrane space (e.g. cytochrome c and AIF), which trigger apoptosis<sup>[4,8,9]</sup>. Secondly, mitochondrial membrane permeabilization can induce the release of cytochrome c and other cytotoxic proteins without any rupture of the mitochondrial outer membrane<sup>[8,10]</sup>. This scenario requires the formation of pores within this membrane thanks to the association of two proapoptotic proteins belonging to the Bcl-2 family, namely Bak (already located in the outer membrane) and Bax (which is recruited from the cytosol)<sup>[4,8]</sup>. Importantly, mitochondrial outer membrane permeabilization through the formation of Bax/ Bak pores is not sensitive to cyclosporine-A<sup>[11,12]</sup>. Thus, whatever the mechanism involved in membrane permeabilization, this event can strongly alter mitochondrial function and structure, and thus lead to cell death. Finally, it is noteworthy that the MPTP structure seems to be different from one tissue to another. This may explain why some organs could be more or less vulnerable to certain permeability transition inducers<sup>[13,14]</sup>.

### **Mitochondrial production of reactive oxygen species**

A major feature of the mitochondria is the production of reactive oxygen species (ROS) through the activity of the mitochondrial respiratory chain (MRC)<sup>[15,16]</sup>. Indeed, a small fraction of electrons entering the MRC can prematurely escape from complexes I and III and directly react with oxygen to generate the superoxide anion radical. This radical is then dismutated by the mitochondrial manganese superoxide dismutase (MnSOD) into hydrogen

peroxide ( $H_2O_2$ ), which is detoxified into water by the mitochondrial glutathione peroxidase (GPx) that uses reduced glutathione (GSH) as a cofactor. Hence, in the normal (non-diseased) state, most of the ROS generated by the MRC are detoxified by the mitochondrial antioxidant defenses. The remaining (i.e. non-detoxified) ROS diffuse out of mitochondria and serve as second messengers to trigger cellular processes such as mitogenesis<sup>[15]</sup>. However, this detoxification process can be overwhelmed in different pathophysiological circumstances. This occurs in particular in case of GSH depletion within liver mitochondria, which reduces greatly their capability to detoxify  $H_2O_2$  since they do not have catalase<sup>[17]</sup>. Depletion of mitochondrial GSH below a critical threshold thus favours  $H_2O_2$  accumulation by impairing its detoxification. This in turn triggers mitochondrial dysfunction, MPTP opening, activation of c-Jun-N-terminal kinase (JNK), and cell death.<sup>[18, 19]</sup> Chronic ethanol intoxication, fasting, and malnutrition are diseased states favouring GSH depletion, in particular within mitochondria. Mitochondrial anti-oxidant enzymes can also be overwhelmed when MRC is chronically impaired. Indeed, a partial block in the flow of electrons greatly increases the probability of mono-electronic reduction of oxygen and superoxide anion production within the complexes I and III<sup>[20, 21]</sup>. High steady state levels of ROS then damage OXPHOS proteins, cardiolipin, and mtDNA<sup>[22-24]</sup>. This oxidative damage aggravates mitochondrial dysfunction to further augment electron leakage and ROS formation, thus leading to a vicious circle.<sup>[25]</sup>

#### DRUG-INDUCED MITOCHONDRIAL DYSFUNCTION AND LIVER INJURY

1. Drug-induced adverse events and mitochondrial toxicity  
The view that drugs could disturb mitochondrial function emerged several decades ago when clinical studies reported in some medicated individuals the occurrence of symptoms usually

observed in patients presenting a mitochondrial disease of genetic origin or a Reye's syndrome (whose physiopathology involves severe mitochondrial dysfunction)<sup>[26]</sup>. Likewise, myopathy, lactic acidosis, and hepatic steatosis have been reported in the late 80's and early 90's in patients treated with the antiretroviral nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine (AZT), zalcitabine (ddC), didanosine (ddl) and stavudine (d4T)<sup>[26-29]</sup>. Since then, the list of drugs inducing adverse events due to mitochondrial dysfunction has not ceased to grow year after year. Regarding drug-induced liver diseases, different mechanisms of mitochondrial dysfunction have been described thus far, including membrane permeabilization, OXPHOS impairment, FAO inhibition, and mtDNA depletion<sup>[5, 6, 26]</sup>. Importantly, DILI due to mitochondrial toxicity has led to the interruption of clinical trials, or drug withdrawal after marketing, in particular when the benefit/risk ratio was deemed to be too low for the patient's healthiness. Moreover, some marketed drugs have received Black Box warnings from drug agencies due to mitochondrial dysfunction and related hepatotoxicity<sup>[5, 30]</sup>.

#### DRUG-INDUCED MPTP OPENING

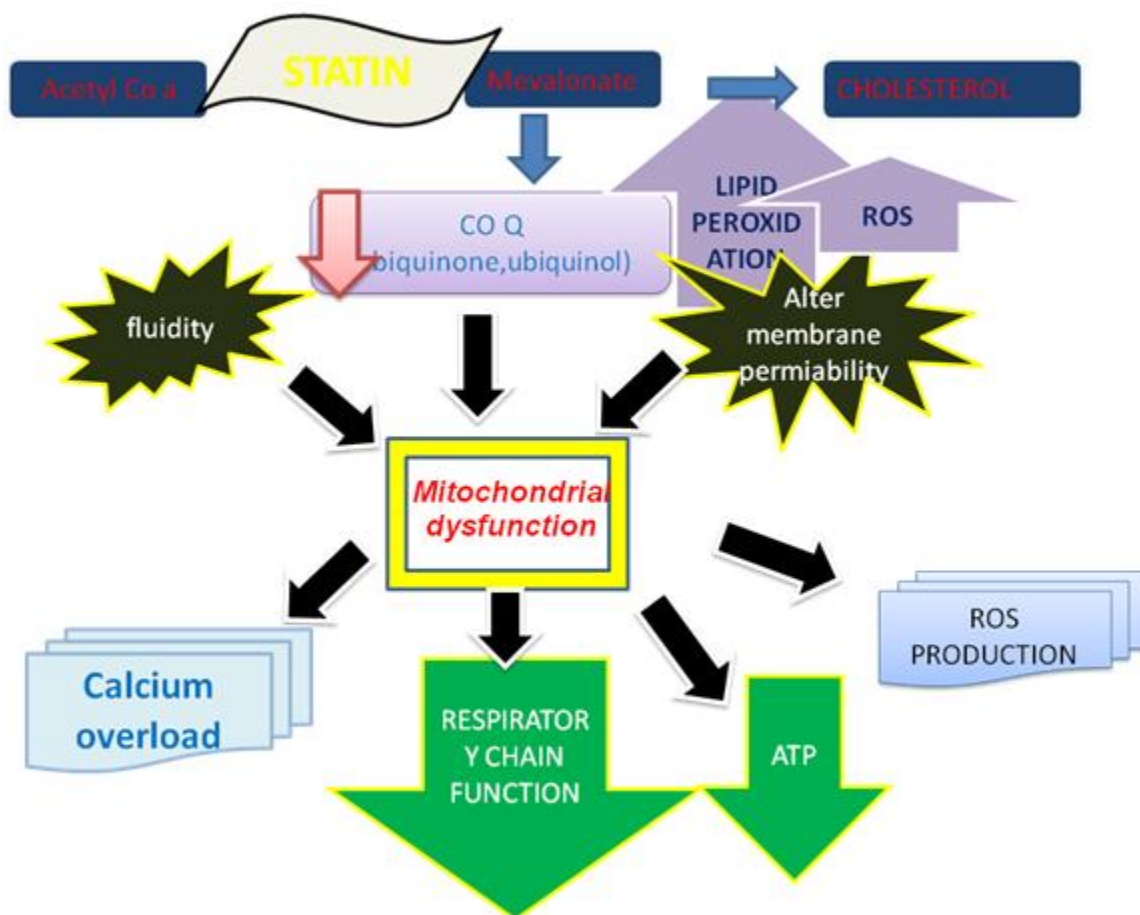
MPTP opening is one mechanism whereby drugs can induce cytolytic hepatitis.<sup>[5, 11, 31-35]</sup> Among these drugs, disulfiram can also induce mitochondrial membrane permeabilization through a MPTP-independent mechanism<sup>[11]</sup>. The precise mechanisms whereby drugs can induce MPTP opening are not known although recent investigations suggest at least three hypotheses, which are not mutually exclusive. Firstly, drugs can interact with some MPTP components<sup>34</sup>. Secondly, drug-induced oxidative stress can favor the oxidation of regulatory thiol groups located within some MPTP components<sup>[11, 36-40]</sup>. Thirdly, drugs such as APAP and cisplatin could cause mitochondrial permeability transition through activation of

JNK or other endogenous MPTP inducers. [19, 38, 41, 42]

### Mechanisms of Atorvastatin - induced impairment of mitochondrial permeability and Co enzyme Q level.

Statins are potent lipid-lowering drugs which reduce the concentrations of low density lipoprotein (LDL) cholesterol. Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase - a rate limiting enzyme in cholesterol biosynthesis - which converts HMG-CoA to mevalonate. [43] Mevalonate is a precursor of

cholesterol but also of a whole class of other important substances, such as ubiquinone, dolichols and other isoprenoids [44]. Statins are not free from side effects despite being considered as safe drugs. [45] Although infrequent, hepatotoxicity and myopathy are two of the most common complications associated with this class of drugs, especially when used at maximum doses, or when combined with other lipid lowering drugs such as fibrates, or combined with drugs that use the same enzymatic pathway as cytochrome P450 (CYP450) in its metabolic pathway, or in the elderly, or in subjects having considerable hepatic and/or renal dysfunction. [45]



Many studies have demonstrated that statins decrease CoQ concentration in plasma and various tissues of experimental animals. Atorvastatin administered for only 14 days decreased plasma CoQ by about 50% in patients with hypercholesterolemia<sup>[43]</sup>. Ubiquinone depletion induced by statin therapy may be accompanied by impaired mitochondrial function, as evidenced by reduced oxygen consumption and reduced capacity of the respiratory chain and rate of ATP synthesis in the liver mitochondria. Thus, both CoQ levels (and thus antioxidant defense) and membrane lipid composition, which might depress oxygen consumption and energy production in the mitochondria.<sup>[46]</sup>

In physiological conditions mitochondrial function are an index of uptake of oxygen, calcium handling, production of ATP, and

regulation of respiratory chain function. Mitochondrial lipid peroxidation alters membrane integrity and permeability of mitochondria. Thus alteration of Atorvastatin result in modulation of mitochondrial function leads to irreversible liver injury.<sup>[44]</sup>

### CONCLUSION

Mitochondrial functions are an index of uptake of oxygen, calcium handling, and production of ATP and regulation of respiratory chain function in physiological conditions. Mitochondrial lipid peroxidation alters membrane integrity and permeability of mitochondria. Thus modulation of mitochondrial function owing to Atorvastatin leads to irreversible liver injury.

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