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# Alteration of mitochondrial DNA homeostasis in drug-induced liver injury

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## Abstract

Mitochondrial DNA (mtDNA) encodes for 13 proteins involved in the oxidative phosphorylation (OXPHOS) process. In liver, genetic or acquired impairment of mtDNA homeostasis can reduce ATP output but also decrease fatty acid oxidation, thus leading to different hepatic lesions including massive necrosis and microvesicular steatosis. Hence, a severe impairment of mtDNA homeostasis can lead to liver failure and death. An increasing number of investigations report that some drugs can induce mitochondrial dysfunction and drug-induced liver injury (DILI) by altering mtDNA homeostasis. Some drugs such as ciprofloxacin, antiretroviral nucleoside reverse-transcriptase inhibitors and tacrine can inhibit hepatic mtDNA replication, thus inducing mtDNA depletion. Drug-induced reduced mtDNA levels can also be the consequence of reactive oxygen species-mediated oxidative damage to mtDNA, which triggers its degradation by mitochondrial nucleases. Such mechanism is suspected for acetaminophen and troglitazone. Other pharmaceuticals such as linezolid and tetracyclines can impair mtDNA translation, thus selectively reducing the synthesis of the 13 mtDNA-encoded proteins. Lastly, some drugs might alter the mtDNA methylation status but the pathophysiological consequences of such alteration are still unclear. Drug-induced impairment of mtDNA homeostasis is probably under-recognized since preclinical and post-marketing safety studies do not classically investigate mtDNA levels, mitochondrial protein synthesis and mtDNA oxidative damage.

## 1. Introduction

More than 350 drugs have been reported to induce liver injury such as hepatic cytolysis, cholestasis, steatosis (i.e. fat accretion) and steatohepatitis, or less frequently cirrhosis and liver cancer (Biour et al., 2004; Björnsson and Hoofnagle, 2016). In the most severe cases, drug-induced liver injury (DILI) can require liver transplantation, or lead to the death of the patient (Fromenty and Pessayre, 1995; Gulmez et al., 2018). It is now acknowledged that mitochondrial dysfunction is a major mechanism whereby drugs can induce DILI (Labbe et al., 2008; Porceddu et al., 2012; Ramachandran et al., 2018), although other mechanisms can be involved such as endoplasmic reticulum (ER) stress (Foufelle and Fromenty, 2016) and lysosomal alterations (Woolbright et al., 2012).

Most of the time, investigations on drug-induced mitochondrial dysfunction report the acute effects of pharmaceuticals on different mitochondrial parameters such as membrane potential and permeability, oxygen consumption, activity of different complexes of the respiratory chain and flux of fatty acid oxidation (Labbe et al., 2008; Nadanaciva and Will, 2011; Porceddu et al., 2018). However, there is an increasing number of investigations reporting that drugs can induce mitochondrial dysfunction and DILI by impairing mitochondrial DNA (mtDNA) homeostasis, for instance at the level of mtDNA replication and translation (Begriche et al., 2011; Igoudjil et al., 2006; Will and Dykens, 2014). Such mechanism of mitochondriotoxicity is most probably under-recognized because preclinical and post-marketing safety studies do not classically investigate drug-induced effects on mtDNA integrity and maintenance.

In this review, I first recall the main features of mitochondria, their role in energy production and the mitochondrial genome, in particular regarding its replication, transcription, translation and repair. Next, drug-induced impairment of mtDNA homeostasis is discussed in separate sections depending on the involved mechanism including inhibition of mtDNA replication, impairment of mtDNA translation and oxidative damage to mtDNA.

## 2. Main features of the mitochondria

### 2.1. Structure and main components

Mitochondria, whose size is  $\sim 1 \mu\text{m}$ , are intracellular organelles with two membranes (namely the outer and the inner membrane) that surround the matrix. This compartment contains numerous enzymes involved in different key oxidative pathways such as mitochondrial fatty acid oxidation (mtFAO) and pyruvate oxidation via the tricarboxylic acid (TCA) cycle. The matrix also contains the mitochondrial DNA (mtDNA) and all the components (e.g. enzymes and transcription factors) mandatory for its replication, transcription, translation and repair. However, this small genome allows only the synthesis of 13 polypeptides of the mitochondrial respiratory chain (MRC), whereas all other mitochondrial proteins ( $\sim 1700$ ) are encoded by the nuclear DNA and targeted to mitochondria (Area-Gomez and Schon, 2014; Borst and Grivell, 1981). Notably, mitochondria share different major features with bacteria including size, double-membrane structure, mode of mtDNA transcription and some components of the mtDNA translation machinery (Santini et al., 2017; Schon and Fromenty, 2015). These features are due to the fact that the precursors of mitochondria were bacteria that became endosymbiotic residents of “proto-eukaryotic” cells early in our evolutionary history (Pessayre et al., 2010).

### 2.2. Production of energy

One major role of mitochondria is the production of energy so that these organelles are often referred to as the powerhouses of the cells. To this end, mitochondria oxidize many substrates including amino acids, pyruvate and fatty acids. Pyruvate, which is provided by glycolysis, is oxidized by the TCA cycle after its transformation into acetyl-CoA by the mitochondrial enzyme

pyruvate dehydrogenase. TCA cycle includes 8 enzymes including the FAD-dependent succinate dehydrogenase (SDH, also referred to as complex II of the MRC) and 3 different NAD<sup>+</sup>-dependent dehydrogenases. Fatty acids are oxidized by the  $\beta$ -oxidation process, a key metabolic pathway mandatory for the preservation of normal energy output, especially during fasting. Two reactions of mtFAO are catalyzed by different FAD-dependent and NAD<sup>+</sup>-dependent dehydrogenases that have specific activities for short-chain, medium-chain, long-chain and very long-chain fatty acids (Fromenty and Pessayre, 1995; Houten et al., 2016).

Substrate oxidation within mitochondria is coupled to the generation of ATP in most normal physiological conditions. Indeed, oxidation of substrates such as pyruvate and fatty acids constantly generate FADH<sub>2</sub> and NADH. These reduced cofactors then transfer their electrons to the MRC, thus regenerating the NAD<sup>+</sup> and FAD necessary for other cycles of fuel oxidation. The electrons provided by NADH or FADH<sub>2</sub> migrate all the way along the respiratory chain, up to cytochrome *c* oxidase (COX), where they safely react with oxygen and protons to form water. Notably, electron transfer across MRC complexes I, III and IV is coupled with the extrusion of protons from the mitochondrial matrix into the intermembrane space of mitochondria, thus creating a large electrochemical potential ( $\Delta\psi$ ) across the inner membrane. When ADP is high, protons reenter the matrix through the F<sub>0</sub> portion of ATP synthase, causing the conversion of ADP into ATP by the F<sub>1</sub> portion of this enzyme (Begrache et al., 2013; Wallace et al., 2010). The biochemical process linking substrate oxidation to ATP generation is referred to as oxidative phosphorylation (OXPHOS).

### *2.3. Production of reactive oxygen species and their deleterious effects*

Mitochondria are considered as the main source of reactive oxygen species (ROS) in most cells. Although the MRC complexes I and III are two major sites of ROS generation in mitochondria (Begrache et al., 2013; Wallace et al., 2010), some enzymes of the mtFAO pathway could also produce significant amount of ROS (Kakimoto et al., 2015; Seifert et al., 2010). Physiological

production of mitochondrial ROS could serve as key signaling molecules (Diebold and Chandel, 2016). Fortunately, different mitochondrial enzymes can eliminate ROS such as manganese superoxide dismutase (MnSOD), peroxiredoxins and glutathione peroxidases, which necessitate reduced glutathione (GSH) as cofactor (Pessayre et al., 2010; Wallace et al., 2010). Hence, mitochondrial GSH plays a key role in order to avoid the excessive accumulation of ROS and subsequent oxidative stress (Ribas et al., 2014).

In contrast, ROS overproduction can induce oxidative damage to different mitochondrial components, including lipids such as cardiolipin, proteins involved in MRC activity or other metabolic processes and mtDNA (Musatov and Robinson, 2012; Sharma and Sampath, 2019). Regarding the mitochondrial genome, ROS can induce directly or indirectly the occurrence of oxidized bases such as 8-hydroxydeoxyguanosine (8-OH-dG), abasic sites (i.e. DNA sites lacking a purine or pyrimidine base), point mutations, deletions, strand breaks and also reduced mtDNA levels, as discussed later on (Anson et al., 1998; Berneburg et al., 1999; Sharma and Sampath, 2019). Notably, the accumulation of oxidative damage in cardiolipin, MRC proteins and mtDNA can impair the activity of different MRC complexes including complexes I and III (Musatov and Robinson, 2012), thus reinforcing ROS overproduction via a vicious circle (Labbe et al., 2008).

#### *2.4. Mitochondrial biogenesis*

Mitochondrial biogenesis is a general program aimed at producing new functional mitochondria with intact mtDNA copies in response to different types of stress. For instance, reduced ATP production and oxidative stress are able to trigger mitochondrial biogenesis (Gureev et al., 2019; Ploumi et al., 2017). The regulation of mitochondrial biogenesis is complex and orchestrated by different key transcription factors and coactivators including nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 $\alpha$  and 1 $\beta$  (PGC-1 $\alpha$  and PGC-1 $\beta$ ) (Ploumi et al., 2017; Villena, 2015). The nuclear factor erythroid 2-like 2 (NFE2L2, also referred

to as Nrf2) also plays a major role in mitochondrial biogenesis in response to oxidative stress (Gureev et al., 2019; Ploumi et al., 2017). These transcription factors and coactivators enhance the expression of numerous nuclear DNA-encoded mitochondrial proteins as well as the mitochondrial transcription factor A (TFAM), which in turn activates mtDNA transcription (Ploumi et al., 2017; Scarpulla et al., 2012). Finally, it is noteworthy that AMP-activated protein kinase (AMPK) plays also a key role in mitochondrial biogenesis, in particular by activating PGC1 $\alpha$  (Vega et al., 2015; Ploumi et al., 2017).

### 3. The mitochondrial genome

#### 3.1. General features of mtDNA

Mammalian mtDNA is a 16.6 kb double-stranded circular genome present within each mitochondrion in several copies. The double-stranded structure of mtDNA consists of a guanine-rich heavy (H) strand and a light (L) strand. Notably, mtDNA encodes 13 polypeptides of the mitochondrial respiratory chain (MRC) (Schon and Fromenty, 2015; Wallace et al., 2010). These polypeptides are then inserted in the inner membrane within the MRC complexes I, III, IV (cytochrome *c* oxidase) and V (ATP synthase), along with dozens of nuclear DNA-encoded proteins. mtDNA also encodes 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) that are required for protein synthesis in mitochondria (Borst and Grivell, 1981). Hence, mtDNA is mandatory for correct MRC activity, OXPHOS and ATP synthesis (Fig. 1).

Although mtDNA is not protected by histones, this genome is entirely coated by numerous molecules of TFAM (Sharma and Sampath, 2019; Farge and Falkenberg, 2019). Hence, in addition to its role in mtDNA transcription (section 2.4), TFAM might also protect the mitochondrial genome against oxidative stress (Kang et al., 2007). mtDNA, TFAM and other proteins such as mtSSB (mitochondrial single-stranded DNA-binding protein) and DNA polymerase gamma ( $\gamma$ ) are organized



into nucleoprotein complexes known as nucleoids, which appear to be associated with the inner mitochondrial membrane (Sharma and Sampath, 2019; Farge and Falkenberg, 2019).

In the normal situation, all the mtDNA copies in a tissue are identical, a situation known as homoplasmy. However, in some pathophysiological conditions (e.g. mitochondrial diseases, aging, alcoholic liver diseases), both normal and mutated mtDNA copies may coexist within the same tissue, a situation known as heteroplasmy. Notably, most mtDNA-based diseases are functionally "recessive", so that the mutant load should be very high (e.g.  $\geq 80\%$ ) before clinical pathology ensues (Schon and Fromenty, 2015). It is also important to bear in mind that there is an excess of mtDNA molecules in most tissues including liver. Hence, a small or moderate loss of mtDNA copies has no deleterious impact on mitochondrial function. It is deemed that the number of mtDNA copies must fall below 20 to 40% of basal levels to induce severe mitochondrial dysfunction and adverse events (Begrache et al., 2011; Ducluzeau et al., 2002; Rossignol et al., 2003). In this context, the few mtDNA copies remaining within each mitochondrion are not able to provide enough MRC polypeptides, thus leading to OXPHOS impairment (Fig. 1). Reduced MRC activity can also lead to secondary inhibition of mtFAO and TCA cycle (Fig. 1), due to a lower mitochondrial content of  $\text{NAD}^+$  and FAD generated respectively from NADH or  $\text{FADH}_2$  (Begrache et al., 2011; Fromenty, 2019). Finally, it is noteworthy that mtDNA can be methylated although the mechanisms of methylation and demethylation in mitochondria are still poorly understood (Sharma and Sampath, 2019; Sharma et al., 2019). Similar to nuclear DNA, mtDNA methylation strongly regulates gene expression (Pirola et al., 2013; Sharma and Sampath, 2019).

### *3.2. mtDNA replication, transcription and translation*

Replication of the mitochondrial genome occurs not only in dividing cells but also in post-mitotic tissues. Permanent mtDNA replication in non-dividing cells allows to keep constant cellular mtDNA levels despite continuous degradation of the most damaged and/or dysfunctional mitochondria. The

mtDNA replication machinery involves different proteins including DNA polymerase  $\gamma$ , mtSSB and Twinkle, a mitochondrial DNA helicase (Farge and Falkenberg, 2019; Sharma and Sampath, 2019). Two mitochondrial topoisomerases, namely Top1mt and Top2 $\beta$ , also regulate mtDNA replication (Goffart et al., 2019). Several models of mtDNA replication have been proposed, although the "asynchronous, strand displacement" model is the most accepted so far (Schon and Fromenty, 2015; Sharma and Sampath, 2019).

Transcription of human mtDNA is "prokaryotic-like". Indeed, all 37 genes encoded by human mtDNA are synthesized initially as two large polycistronic precursor transcripts, respectively encoded by the H-strand and the L-strand. The tRNA genes are then cleaved at their 5' and 3' ends by two ribonucleases, thereby releasing the tRNAs as well as the flanking rRNAs and mRNAs (Schon and Fromenty, 2015; Sharma and Sampath, 2019). Following cleavage of the precursor polycistronic RNAs, the 3' termini of the mRNAs are polyadenylated. Translation of the mature mRNAs takes place on mitochondrial ribosomes, which consist of mtDNA-encoded 12S and 16S rRNAs together with different imported ribosomal proteins (Christian and Spremulli, 2012; Schon and Fromenty, 2015).

### 3.3. mtDNA repair

Similarly to nuclear DNA, the mitochondrial genome can undergo different types of damage, which require specific pathways of DNA repair in order to preserve mitochondrial function. Indeed, contrary to what has been proposed several decades ago, mitochondria do possess several of the DNA repair pathways described for the nuclear genome including a base excision repair (BER) system, a mismatch repair (MMR)-like activity, non-homologous end joining (NHEJ) and possibly homologous recombination (Sharma and Sampath, 2019; Sharma et al., 2019; Zinovkina, 2018). Furthermore, some components of the nucleotide excision repair (NER) pathway are present in mitochondria (Sharma et al., 2019; Zinovkina, 2018). Hence, these organelles are expected to repair most types of DNA damage

including oxidized DNA bases, abasic sites, double-strand breaks and bulky DNA adducts (Muftuoglu et al., 2014; Sharma et al., 2019; Valente et al., 2016; Zinovkina, 2018).

Besides the above-mentioned DNA repair pathways, it should be underlined that mitochondria are able to completely degrade any mtDNA molecules that present too many DNA lesions to be efficiently repaired, in particular double-strand breaks and possibly bulky DNA adducts (Moretton et al., 2017; Valente et al., 2016). This degradation seems to be dependent on several enzymes including MGME1 (mitochondrial genome maintenance exonuclease 1) and DNA polymerase  $\gamma$ , which possesses an exonuclease activity (Nissanka et al., 2018; Peeva et al., 2018). Notably, the nuclease-mediated degradation of heavily damaged mtDNA molecules can cause rapid mtDNA depletion (Nissanka et al., 2018; Peeva et al., 2018). This mechanism of mtDNA depletion has been proposed to explain the severe loss of mtDNA copies observed in liver after an alcoholic binge (Mansouri et al., 1999; Mansouri et al., 2001), or carbon tetrachloride (CCl<sub>4</sub>) intoxication (Knockaert et al., 2012).

If mtDNA degradation is not complete, partially degraded mtDNA molecules can generate mtDNA deletions after NHEJ-mediated DNA ligation (Nissanka et al., 2019). However, it should be underscored that mtDNA deletions can also arise from errors in mtDNA replication such as slipped mispairing (Chen et al., 2011; Nissanka et al., 2019). Slipped-mispairing replication generates large mtDNA deletions that are usually flanked by small direct DNA repeats with identical sequences (Chen et al., 2011; Nissanka et al., 2019). Interestingly, oxidative stress greatly favors the occurrence of such large mtDNA deletions in human liver, for instance in the context of normal aging, Wilson's disease and alcoholic intoxication (Fromenty et al., 1995; Krishnan et al., 2008; Mansouri et al., 1997; Simonetti et al., 1992). However, in these pathophysiological conditions, mtDNA deletions are present in liver only at low heteroplasmy levels (<0.1%) (Fromenty et al., 1995; Mansouri et al., 1997; Simonetti et al., 1992). Hence, these mtDNA deletions are most likely not pathogenic as such but may rather reflect oxidative damage of the mitochondrial genome (Schon and Fromenty, 2015).

Finally, it is noteworthy that mtDNA repair requires DNA polymerases other than DNA polymerase gamma. For instance, it has been shown that DNA polymerase beta and PrimPol, a DNA

primase-polymerase, are able to participate to mtDNA repair in addition to their usual role in nuclear DNA repair (Kaufman and Van Houten, 2017; Torregrosa-Muñumer et al., 2017). DNA polymerases theta and zeta could also play a significant role in mtDNA maintenance (Sharma et al., 2019).

#### 4. Hepatotoxic drugs impairing mtDNA homeostasis

##### 4.1. General overview

The antiretroviral nucleoside reverse-transcriptase inhibitor (NRTI) zidovudine (AZT) was the first pharmaceutical found to induce hepatotoxicity in treated patients through an impairment of mtDNA replication, resulting in severe mtDNA depletion in liver (Chariot et al., 1999; Le Bras et al., 1994). Since then, other hepatotoxic drugs have been reported to alter mtDNA homeostasis not only by inhibiting mtDNA replication but also by impairing mtDNA translation (Fig. 2) (Fromenty, 2019; Schon and Fromenty, 2015). Whatever its mechanism, drug-induced impairment of mtDNA replication and translation in liver can lead to hepatic cytolysis with increased plasma transaminases, steatosis, steatohepatitis and cirrhosis (Fig. 1) (Chariot et al., 1999; Margolis et al., 2014; Schon and Fromenty, 2015). Notably, steatosis can occur as microvesicular fat, thus reflecting profound inhibition of mtFAO (Fromenty, 2019; Schon and Fromenty, 2015). Hyperlactatemia or lactic acidosis (sometimes fatal), can also occur in some patients, thus reflecting an impairment of the TCA cycle (Margolis et al., 2014; Schon and Fromenty, 2015). Hence, severe impairment of mtDNA replication or translation can reduce MRC activity and secondarily curb the mitochondrial oxidation of different endogenous molecules such as fatty acids and pyruvate, which respectively accumulate as triglycerides and lactate (Margolis et al., 2014; Schon and Fromenty, 2015). The progression of steatosis towards steatohepatitis in some treated patients could be explained by oxidative stress and lipid peroxidation, which favor the occurrence of inflammation and fibrogenesis (Allard et al., 2019; Massart et al., 2017; Satapathy et al., 2015). Finally, it is noteworthy that impairment of MRC activity and subsequent ATP shortage can not

only explain hepatocyte necrosis (and thus hepatic cytolysis) but also cholestasis (Fig. 1), because the activity of several bile transporters is ATP dependent (Aleo et al., 2014; Borgne-Sanchez and Fromenty, 2018).

For some drugs such as acetaminophen and troglitazone, mtDNA depletion is not linked to an impairment of mtDNA replication but rather to mtDNA oxidative damage (Fig. 2) (Fromenty, 2019; Schon and Fromenty, 2015). Indeed, ROS can induce different types of DNA lesions such as single and double-strand breaks that can trigger complete mtDNA degradation by different mitochondrial enzymes including MGME1 and DNA polymerase  $\gamma$ , as previously mentioned (Nissanka et al., 2018; Peeva et al., 2018). Although the resulting mtDNA depletion could have a pathogenic role, the main mechanism whereby these drugs induce mitochondrial dysfunction is rather a direct impairment of mitochondrial function including MRC activity and OXPHOS, as discussed below. Hence, similar to alcohol intoxication (Cahill et al., 1999; Mansouri et al., 1999; Mansouri et al., 2001), APAP and troglitazone-induced mtDNA depletion reflects the occurrence of massive oxidative stress in liver mitochondria.

Although drug-induced impairment of mitochondrial function and mtDNA homeostasis can eventually lead to DILI, propagation (and thus aggravation) of the initial liver injury can occur through different adaptive or innate immune responses. Readers are invited to read different excellent reviews regarding the role of immunity in idiosyncratic DILI (Roth et al., 2017; Woolbright and Jaeschke, 2018; Ye et al., 2018).

In the following sections, I present the main hepatotoxic drugs that have been shown to alter mtDNA homeostasis by inhibiting mtDNA replication or mtDNA translation as well as by inducing oxidative mtDNA damage. In the last section, other potential mechanisms of drug-induced impairment of mtDNA homeostasis are finally discussed.

#### *4.2. Drugs altering mtDNA replication*

#### 4.2.1. Ciprofloxacin

Ciprofloxacin is a 4-quinolone antibiotic inhibiting the bacterial DNA gyrases, which belongs to the type II topoisomerase family. Ciprofloxacin can induce extensive hepatocellular cytolysis (with possible liver failure), steatosis and cholestasis (Biour et al., 2004; Orman et al., 2011; Wang et al., 2013). In cultured cells, ciprofloxacin progressively decreases mtDNA levels and impair mitochondrial respiration (Lawrence et al., 1993). In addition, further *in vitro* investigations suggested that ciprofloxacin could impair mtDNA replication by inhibiting the type II topoisomerase present in mitochondria (Lawrence et al., 1996). Ciprofloxacin might also induce hepatotoxicity via the production of free radicals and lipid peroxidation, independently of mtDNA depletion (Gürbay et al., 2001; Weyers et al., 2002). A recent study also reported that ciprofloxacin (30 to 210  $\mu$ M) was able to induce the opening of the mitochondrial membrane permeability transition (MPT) pore and mitochondrial swelling in isolated rat liver mitochondria (Oyebode et al., 2019). However, such concentrations of ciprofloxacin failed to induce mitochondrial swelling in isolated mouse liver mitochondria (Porceddu et al., 2012).

#### 4.2.2. Fialuridine

The nucleoside analogue 1-(2-deoxy-2-fluoro- $\beta$ -d-arabinofuranosyl)-5-iodouracil (FIAU), or fialuridine, has been developed in the early 90s for the treatment of chronic hepatitis B. However, while preclinical safety studies and a short pilot study in patients did not reveal any signs of hepatotoxicity, phase II clinical trials conducted by the National Institute of Health were prematurely interrupted during the 13<sup>th</sup> week due to serious adverse effects (Attarwala, 2010; Mak et al., 2016). Indeed, FIAU induced in several patients unmanageable lactic acidosis, microvesicular steatosis and liver failure requiring liver transplantation, or leading to death (Fromenty and Pessayre, 1995; Honkoop et al., 1997; McKenzie et al., 1995). New *in vivo* and *in vitro* investigations after this

unexpected tragedy showed that FIAU strongly inhibits DNA polymerase  $\gamma$  and triggers mtDNA depletion by an unusual mechanism (Lewis et al., 1996; Lewis et al., 1997; Semino-Mora et al., 1997). Indeed, unlike NRTIs whose incorporation into a growing chain of mtDNA interrupts mtDNA replication (section 4.2.4), FIAU can be incorporated into mtDNA without immediately blocking mtDNA replication. This is because FIAU carries a 3'-hydroxyl group on the sugar moiety, thus allowing the subsequent incorporation of other nucleotides by the mitochondrial DNA polymerase  $\gamma$ . However, when several adjacent molecules of FIAU are successively incorporated into a growing chain of mtDNA, DNA polymerase  $\gamma$  is strongly inhibited. This subsequently blocks further mtDNA replication and leads to severe mtDNA depletion in liver (Lewis et al., 1996).

The fact that FIAU hepatotoxicity was not detected in preclinical safety studies is particularly puzzling. A likely explanation is that FIAU was unable to inhibit mtDNA replication in the common animal species used in these studies, in particular rats and dogs (Labbe et al., 2008). In contrast, FIAU was able to induce hepatic mtDNA depletion in woodchucks (*Marmota monax*) due to the high rate of incorporation of this drug within the woodchuck mitochondrial genome (Labbe et al., 2008; Lewis et al., 1997). Interestingly, the woodchuck model was chosen afterwards in order to evaluate the mitochondrial toxicity of any novel anti-HBV nucleoside analogues (Attarwala, 2010). Previous investigations suggested that interspecies differences in the susceptibility toward FIAU-induced hepatotoxicity could be related to the subcellular expression of the equilibrative nucleoside transporter 1 (ENT1) (Lee et al., 2006), but other hypotheses were recently put forward (Hendriks et al., 2019). Finally, the FIAU tragedy ghoulishly illustrates the fact that mitochondrial toxicity linked to mtDNA replication impairment can occur long after treatment initiation when the mtDNA reserve becomes too low to allow proper mitochondrial function (section 3.1).

#### 4.2.3. Ganciclovir



The nucleoside analogue 9-(1,3-dihydroxy-2-propoxymethyl) guanine, or ganciclovir, is an antiviral drug effective on different viruses including herpes simplex viruses, varicella-zoster virus, cytomegaloviruses and Epstein-Barr virus. Ganciclovir is actually a prodrug that is transformed in the infected cells into ganciclovir-triphosphate, the active form of ganciclovir. This phosphorylation is preferentially carried out in the infected cells because non-infected cells are less able to perform the first phosphorylation step. Ganciclovir triphosphate exerts its antiviral activity by impairing the synthesis of viral DNA by two mechanisms: competitive inhibition of the viral DNA polymerases and direct incorporation into the viral DNA, which slows down its elongation. Indeed, ganciclovir has two hydroxyl groups so that its incorporation into DNA does not terminate DNA replication. Nonetheless, the incorporated ganciclovir molecules are expected to distort the DNA helix and impede the next round of DNA replication (Foti et al., 1997; Thust et al., 2000). Hepatic cytolysis, cholestasis and diffuse fibrosis have been reported in some patients treated by this drug (Biour et al., 2004; Shea et al., 1987; Wang et al., 2013).

Ganciclovir can also be administered for the treatment of different cancers in association with an adenoviral vector harboring the gene encoding the herpes simplex virus thymidine kinase (HSVtk), a so-called “suicide gene” (Düzgüneş, 2019). The principle of this combined therapy is the HSVtk-mediated conversion of ganciclovir into ganciclovir-monophosphate, which is further activated by cellular kinases into ganciclovir-triphosphate, thus impairing DNA synthesis and leading to apoptosis of the dividing cancer cells (Düzgüneş, 2019). However, this therapy can lead in treated patients to hepatotoxicity and other side effects such as skin rash, thrombocytopenia and anemia (Immonen et al., 2004; Shalev et al., 2000; Sterman et al., 1998). Interestingly, HSVtk/ganciclovir was found to induce serious hepatic dysfunction in rats with swollen hepatocytes with enlarged nuclei, scant necrosis and apoptosis, microvesicular and macrovacuolar steatosis and infiltration of the liver with hematopoietic and immune-response cells such as macrophages (Herraiz et al., 2003; van der Eb et al., 1998).

Different *in vitro* and *in vivo* studies showed that HSVtk/ganciclovir-induced hepatotoxicity was associated with mtDNA depletion and mitochondrial dysfunction characterized by a profound decrease



in COX activity and hyperlactatemia (Herraiz et al., 2003; van der Eb et al., 2003). Interestingly, some of these investigations showed that ganciclovir is incorporated into mtDNA in addition to nuclear DNA (Herraiz et al., 2003). Thus, it is tempting to speculate that the incorporated ganciclovir molecules may distort the mitochondrial genome and impede its replication, similar to what has been proposed for nuclear DNA (Foti et al., 1997; Thust et al., 2000).

#### 4.2.4. NRTIs

NRTIs were the first family of drugs developed for the treatment of acquired immune deficiency syndrome (AIDS) after it was discovered that these compounds could strongly inhibit the human immunodeficiency virus (HIV) reverse transcriptase (Igoudjil et al., 2006; Margolis et al., 2014). The main NRTIs include stavudine (d4T), zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), lamivudine (3TC) and newer molecules such as abacavir (ABC), emtricitabine (EMT) and tenofovir (TEN) (Fromenty and Pessayre, 1995; Margolis et al., 2014). These drugs are 2',3'-dideoxynucleoside analogues in which the hydroxyl group (-OH) in the 3' position on the sugar ring is replaced by either an hydrogen atom or another group unable to form a phosphodiester bond.

The remarkable benefit of NRTIs is unfortunately overshadowed by the occurrence in a significant number of HIV infected patients of severe adverse effects such as pancreatitis, lactic acidosis, myopathy and liver failure (Fromenty and Pessayre, 1995; Igoudjil et al., 2006; Margolis et al., 2014). NRTI-induced hepatotoxicity is particularly observed with d4T, ddI and AZT. Indeed, these drugs can induce different types of liver lesion including cholestasis, microvesicular and macrovacuolar steatosis, steatohepatitis and cirrhosis (Fromenty, 2019; Fromenty and Pessayre, 1995; Margolis et al., 2014; Wang et al., 2013). The newer NRTIs seem to be less hepatotoxic (Soriano et al., 2008). Notably, the spectrum of NRTI-induced adverse effects is similar to the clinical manifestations observed in patients with genetic OXPHOS disorders (Fromenty and Pessayre, 1995; Chariot et al., 1999).

It is now acknowledged that most of the side effects induced by NRTIs are the consequence of their deleterious effects on the mitochondrial genome. Indeed, these drugs are able to inhibit mtDNA replication, thus inducing profound mtDNA depletion and OXPHOS impairment (Fromenty and Pessayre, 1995; Igoudjil et al., 2006; Margolis et al., 2014). NRTIs act as chain terminators after their incorporation into the growing chain of mtDNA by the DNA polymerase  $\gamma$ . If the NRTI molecule is not removed by the proofreading activity of this DNA polymerase, no other nucleotides can be incorporated because the DNA chain now lacks a 3'-OH end (Igoudjil et al., 2006; Margolis et al., 2014).

The ability of NRTIs to inhibit DNA polymerase  $\gamma$  greatly varies among the analogues. Indeed, *in vitro* studies on purified DNA polymerase  $\gamma$  indicated that the potency of the inhibition is as follows: ddC > ddI > d4T >> 3TC > AZT > ABC (Igoudjil et al., 2006; Soriano et al., 2008). Notably, this order of potency is also observed in human liver as the so-called "D-drugs" (ddC, ddI, d4T) appear to inhibit DNA polymerase  $\gamma$  and induce mtDNA depletion more strongly than other NRTIs (Walker et al., 2004). Interestingly, similar to fialuridine (section 4.2.2), NRTI-induced mtDNA depletion can explain why these drugs can induce liver injury in some patients several weeks or months after treatment initiation (Soriano et al., 2008; Margolis et al., 2014).

In addition to mtDNA depletion, NRTIs are able to induce the occurrence of mtDNA deletions and point mutations, possibly as a consequence of mitochondrial oxidative stress (Igoudjil et al., 2006; Liang et al., 2018; Payne et al., 2011). However, it is still unclear whether these mtDNA mutations play a significant role in NRTI-induced mitochondrial dysfunction. Finally, it is noteworthy that experimental studies showed that some NRTIs such as AZT and d4T can induce mitochondrial and metabolic toxicity through mechanisms unrelated to the inhibition of DNA polymerase  $\gamma$  and mtDNA depletion (Igoudjil et al., 2006; Igoudjil et al., 2007; Igoudjil et al., 2008; Lund et al., 2007).

#### 4.2.5. Tacrine

Tacrine, or tetrahydroaminoacridine, is a reversible cholinesterase inhibitor prescribed for Alzheimer's disease. However, this drug increases plasma ALT activity in 50% of recipients and can induce severe liver injury in some individuals (Berson et al., 1996; Wang et al., 2013). Hepatic steatosis has also been reported (Biour et al., 2004). Tacrine is able to inhibit mtDNA synthesis *in vitro* and *in vivo* and to extensively deplete hepatic mtDNA after chronic treatment in mice (Mansouri et al., 2003; Robertson et al., 1998). Some investigations suggest that tacrine could impede mtDNA replication through topoisomerase inhibition (Mansouri et al., 2003). However, it is noteworthy that another mechanism of mitochondrial toxicity seem to be involved in tacrine-induced liver injury. Indeed, tacrine is able to uncouple OXPHOS, decrease membrane potential and reduce ATP synthesis via a protonophoric mechanism similar to amiodarone and perhexiline (Berson et al., 1996; Park et al., 2015; Robertson et al., 1998).

#### 4.2.6. Tamoxifen

Tamoxifen is an antiestrogenic drug used in the treatment of advanced breast cancer. This anticancer drug can, however, induce acute and chronic liver injury, in particular steatosis, steatohepatitis and cirrhosis (Bruno et al., 2005; Fromenty, 2019; Wang et al., 2013). Tamoxifen was found to induce a progressive reduction in hepatic mtDNA levels after chronic treatment in mice, with a strong mtDNA depletion after 28 days (Larosche et al., 2007). Further *in vitro* investigations suggested that impairment of mtDNA replication could be due to tamoxifen intercalation between mtDNA bases and subsequent topoisomerase inhibition (Larosche et al., 2007). In contrast to these investigations, a 14-day treatment with tamoxifen failed to reduce mtDNA levels in human differentiated hepatoma HepaRG cells (Begrache et al., 2019), whereas the same treatment protocol with the NRTI zalcitabine (ddC) induced a severe mtDNA depletion (Le Guillou et al., 2018). It is possible that a longer treatment with tamoxifen might have been needed in order to induce significant mtDNA depletion in HepaRG cells. Finally, it should be underlined that tamoxifen presents other

mitochondriotoxic effects in particular OXPHOS uncoupling via a protonophoric mechanism and mtFAO inhibition (Cardoso et al., 2001; Larosche et al., 2007).

#### 4.3. Drugs impairing mtDNA translation

##### 4.3.1. Linezolid

The oxazolidinone linezolid is an antibiotic particularly efficient against drug-resistant pathogens including the Gram-positive bacteria *Enterococcus faecium* and *Staphylococcus aureus* as well as *Mycobacterium tuberculosis* (Santini et al., 2017). Like many other antibiotics, linezolid impairs bacterial growth by inhibiting protein synthesis via its binding to bacterial ribosomes (Santini et al., 2017). Moderate to serious liver injury can occur in some patients after several weeks of treatment, with the occurrence of increased plasma transaminases and several types of hepatic lesions such as macrovacuolar and microvesicular steatosis as well as bile duct damages (Fromenty, 2019; De Bus et al., 2010; De Vriese et al., 2006; Vinh and Rubinstein, 2009). Prolonged administration of linezolid can also induce lactic acidosis, myopathy, pancreatitis, stroke-like episodes, neuropathy and renal failure (Fromenty, 2019; Santini et al., 2017; Schon and Fromenty, 2015). Similar to NRTIs (section 4.2.4), the spectrum of linezolid-induced adverse effects is similar to the clinical manifestations observed in patients with genetic defects of OXPHOS (Fromenty and Pessayre, 1995; Vinh and Rubinstein, 2009).

Several investigations support the view that linezolid-induced side effects are secondary to a strong inhibition of mammalian mitochondrial protein synthesis via its binding to mitochondrial ribosomes (Garrabou et al., 2017; McKee et al., 2006; Nagiec et al., 2005; Santini et al., 2017). Indeed, mammalian mitochondrial ribosomes have some similarities with bacterial ribosomes, in particular at the level of the linezolid-binding site (Leach et al., 2007; Santini et al., 2017). Linezolid interaction with the mitochondrial ribosomes is expected to impair the ribosomal binding

of aminoacyl tRNAs (Sun et al., 2019). The subsequent inhibition of mtDNA translation thus reduces the activity of MRC complexes that contain mtDNA-encoded proteins (De Vriese et al., 2006; Garrabou et al., 2007; Le Guillou et al., 2018; Santini et al., 2017). Interestingly, it has been reported that the risk of linezolid-induced mitochondrial dysfunction and secondary adverse effects such as lactic acidosis could be increased by several genetic predispositions such as mutations or polymorphisms in the 12S and 16S rRNA genes of the mitochondrial genome, or the mtDNA haplogroups U and J1 (Fromenty, 2019; Garrabou et al., 2017; Santini et al., 2017). However, further investigations will be needed in order to determine whether this mtDNA polymorphism also increases the risk of linezolid-induced liver injury.

#### 4.3.2. Tetracyclines

Tetracyclines are broad-spectrum antibiotics which prevent bacterial growth by inhibiting bacterial protein synthesis. The tetracycline family includes many compounds including tetracycline, doxycycline, minocycline, methacycline and lymecycline. These drugs can induce different types of liver lesion including cholestasis, necrosis and steatosis, classically as microvesicular fat (Biour et al., 2004; Fromenty, 2019; Wang et al., 2013). Microvesicular steatosis was particularly observed several decades ago when tetracyclines were administered at very high intravenous doses (Fromenty, 2019; Fromenty and Pessayre, 1995). Synthetic tetracyclines such as doxycycline and minocycline are now preferred to tetracycline because of easier dose schedules and faster gastrointestinal absorption when taken with food.

Tetracycline derivatives have been shown to inhibit mtDNA translation by a mechanism similar to linezolid, namely via their binding to the mitochondrial ribosomes (Chukwudi, 2016; McKee et al., 2006; Moullan et al., 2015; Protasoni et al., 2018). The glycylicycline antibiotic tigecycline, a tetracycline analogue, is also able to impair mitochondrial protein synthesis (Skrtić et al., 2011), an effect which could have led to hepatotoxicity, acidosis, progressive kidney failure and death in a

patient treated with this drug for several weeks (Vandecasteele et al., 2018). However, it is noteworthy that tetracyclines can also inhibit mtFAO through a mechanism apparently independent on mtDNA translation impairment (Fréneaux et al., 1988; Labbe et al., 1991). However, the exact mechanism whereby tetracyclines inhibit mtFAO is still unknown.

#### 4.3.3. Other hepatotoxic drugs

Recently, *in vitro* investigations carried out in the human differentiated hepatoma HepaRG cells revealed that the antidepressant imipramine and the antiretroviral protease inhibitor ritonavir reduced the levels of the mtDNA-encoded ND1 and COX2 proteins, whereas the amount of COX4 and other nuclear DNA-encoded mitochondrial proteins was unaffected (Le Guillou et al., 2018). These mitochondrial effects were associated with a significant decrease in complex I levels and activity (Le Guillou et al., 2018). These results strongly suggest that both imipramine and ritonavir are able to impair mtDNA translation, although further investigations would be required to decipher the exact mechanism of this deleterious effect. Actually, these results were totally unexpected because mitochondrial protein synthesis is thought to be impaired only by antibiotics such as linezolid (section 4.3.1) and tetracyclines (section 4.3.2). Finally, in addition to mechanistic studies, supplementary investigations should be carried out to determine whether impaired mtDNA translation could be involved in imipramine and ritonavir-induced liver injury (Biour et al., 2004; Wang et al., 2013).

#### 4.4. Drugs inducing oxidative mtDNA damage

##### 4.4.1. Acetaminophen

Acetaminophen (N-acetyl-*p*-aminophenol, APAP or paracetamol) is one of the most popular drugs used for the management of pain and hyperthermia. Although APAP is usually considered as safe, the

inadvertent or intentional ingestion of an excessive dose of this drug can cause massive hepatocellular necrosis and acute liver failure, which can be fatal (Fromenty et al., 2019; Ramachandran and Jaeschke, 2019). Steatosis, in particular microvesicular fat, can be observed in residual viable hepatocytes (Fromenty et al., 2019). Recent articles thoroughly reviewed the mechanisms whereby this drug can induce severe liver injury (Fromenty et al., 2019; Moles et al., 2018; Ramachandran and Jaeschke, 2019). Briefly, APAP-induced hepatotoxicity is caused by cytochrome P450-mediated generation of the highly reactive metabolite N-acetyl-*p*-benzoquinone imine (NAPQI). Indeed, this electrophilic metabolite depletes hepatic glutathione (GSH), increases cell calcium, alters mitochondrial function, enhances ROS and peroxynitrite generation and activates c-Jun N-terminal kinase, to finally trigger liver cell necrosis (Banerjee et al., 2015; Moles et al., 2018; Ramachandran and Jaeschke, 2019).

APAP induces mitochondrial dysfunction by different mechanisms including NAPQI-mediated inhibition of different MRC complexes and possibly of some components of the mtFAO pathway (Barbier-Torres et al., 2017; Chrøis et al., 2019; Fromenty et al., 2019; Lee et al., 2015). Another mechanism whereby APAP might alter MRC activity is mtDNA depletion. Indeed, investigations in mice showed that hepatic mtDNA levels were rapidly depleted after an APAP overdose, possibly due to the mtDNA damage caused by peroxynitrite and ROS (Aubert et al., 2012; Cover et al., 2005; Du et al., 2017; Ge et al., 2019). Interestingly, one of these studies showed that hepatic levels of 11 of the 13 mtDNA-encoded mRNAs were significantly decreased 24 hours after 300 mg/kg APAP (Ge et al., 2019), probably as a consequence of mtDNA depletion. However, it cannot be excluded that oxidatively damaged mRNAs might have been specifically degraded by mitochondrial RNases (Barchiesi and Vascotto, 2019). Another mechanism of reduced mtDNA-encoded mRNAs might be an impaired transcription of the remaining mtDNA molecules. Indeed, previous investigations reported that powerful free radical initiators such as 2,2'-azobis-(2-amidinopropane) hydrochloride (AAPH) were able to strongly inhibit the *de novo* synthesis of mitochondrial RNAs (Fromenty and Pessayre, 1995; Kristal et al., 1994). It is noteworthy that fragments of mtDNA and nuclear DNA can be



detected in the circulation of patients with APAP-induced liver injury, thus reflecting massive DNA degradation during APAP hepatotoxicity (McGill et al., 2012; McGill et al., 2014). Lastly, it should be mentioned that induction of mitochondrial biogenesis with the sirtuin 1 (SIRT1) activator SRT1720 significantly protected mice against late liver injury after APAP overdose (Du et al., 2017).

#### 4.4.2. Troglitazone

The thiazolidinedione troglitazone was the first peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonist used for the treatment of type 2 diabetes. However, this drug has been withdrawn from the market in 2000 after the occurrence of numerous cases of serious and sometimes fatal liver injury (Fromenty, 2019; Yokoi et al., 2010). In most patients, the histopathologic changes included massive necrosis but other lesions such as cholestasis, microvesicular and macrovacuolar steatosis, fibrosis and cirrhosis have also been reported (Fromenty, 2019; Wang et al., 2013). Previous articles extensively reviewed the mechanisms whereby troglitazone can induce liver injury (Fromenty et al., 2019; Masubuchi et al., 2006; Yokoi et al., 2010). Briefly, troglitazone is able to impair mitochondrial function by different mechanisms including opening of the MPT pore and inhibition of the different MRC complexes (Fromenty, 2019; Nadanaciva et al., 2007; Okuda et al., 2010). Interestingly, troglitazone hepatotoxicity in mice is significantly favored in the context of partial deficiency in MnSOD (encoded by the *sod2* gene) (Lee et al., 2013; Ong et al., 2007), a major mitochondrial antioxidant enzyme (Azadmanesh and Borgstahl, 2018; Degoul et al., 2001). In addition, troglitazone could also indirectly impair MRC activity by inducing mtDNA depletion, possibly as a consequence of oxidative mtDNA damage such as strand breaks (Hu et al., 2015; Rachek et al., 2009). Hence, this mechanism of troglitazone-induced mtDNA depletion seems similar to what has been proposed for APAP (section 4.4.1), ethanol (Mansouri et al., 1999; Mansouri et al., 2001) and CCl<sub>4</sub> (Knockaert et al., 2012).



#### 4.5. Other mechanisms of drug-induced impairment of mtDNA homeostasis

Recently, two studies reported that the antiepileptic drug valproic acid (VPA) disturbed the methylation status of the mitochondrial genome (Wolters et al., 2017; Wolters et al., 2018). More specifically, a 2-day treatment of human hepatocytes with VPA (15 mM) increased the methylation of mtDNA (Wolters et al., 2018), whereas a longer treatment (e.g. 5 days) in the same cellular model was associated with a hypomethylation of the mitochondrial genome (Wolters et al., 2017). However, further investigations will be required in order to determine whether disturbance of the methylation status of the mitochondrial genome can alter mitochondrial function and play a role in VPA-induced liver injury. Although this hypothesis is attractive, it should be reminded that numerous studies showed that VPA-induced hepatotoxicity is mainly the consequence of an impairment of mtFAO *per se* and possibly of MRC activity (Begriche et al., 2011; Fromenty and Pessayre, 1995; Massart et al., 2018). Finally, it is noteworthy that VPA treatment can trigger liver failure in individuals with preexisting mtDNA depletion due to mutations in the human DNA polymerase gamma (POLG) gene (Schon and Fromenty, 2015; Li et al., 2015). This susceptibility is probably secondary to an aggravation of preexisting MRC dysfunction but not of mtDNA depletion by itself. Indeed, *in vitro* investigations reported that VPA was not able to reduce mtDNA levels, although only myoblasts were investigated in this study (Stewart et al., 2010). It was also reported that long-term therapy with different anti-HBV drugs increased mtDNA methylation in peripheral blood mononuclear cells in treated patients (Madeddu et al., 2017). However, no investigation was carried out in liver. Thus, further studies will be necessary in order to determine whether higher mtDNA methylation could play a role in liver injury induced by these drugs.

Lastly, it is noteworthy that drugs may alter hepatic mtDNA levels via a general effect on mitochondrial biogenesis. However, only a few investigations have dealt with this issue. For instance, the antitubercular drug isoniazid was found to impair mitochondrial biogenesis in HepG2 cells via a PGC1 $\alpha$ -dependent pathway but mtDNA levels were not measured in this study (Zhang et

al., 2017). Conversely, other drugs were found to stimulate mitochondrial biogenesis and increase mtDNA levels in cultured hepatocytes. Indeed, non-cytotoxic concentrations of APAP (0.5 and 1 mM) increased the mtDNA copy number in HepG2 cells, an effect that might have been triggered by NFE2L2 (Nrf2) activation (Zhang et al., 2016). We also found that mtDNA levels were enhanced in differentiated HepaRG cells treated with imipramine, amiodarone, carbamazepine, terbinafine and troglitazone, which were used at concentrations inducing slight or no cytotoxicity (Le Guillou et al., 2018). For amiodarone and imipramine, the robust increase in phospho-AMPK $\alpha$  levels and PGC1 $\alpha$  mRNA expression could suggest a *bona fide* activation of mitochondrial biogenesis (Le Guillou et al., 2018). However, we did not identify in this study the cue(s) responsible for AMPK activation such as increased AMP/ATP ratio, ROS and reactive nitrogen species (Cardaci et al., 2012; Sid et al., 2013). For amiodarone, carbamazepine and troglitazone, we also found that the increase in mtDNA levels observed in treated HepaRG cells was significantly blunted when cells were concomitantly incubated with fatty acids (Le Guillou et al., 2018). Hence, these results suggest that adaptive mitochondrial biogenesis triggered by some drugs might be impaired in the context of nonalcoholic liver disease (NAFLD) (Le Guillou et al., 2018). Interestingly, basal hepatic mitochondrial biogenesis seems to be impaired in NAFLD (Aharoni-Simon et al., 2011; Handa et al., 2014).

## 5. Conclusions

Drug-induced impairment of hepatic mtDNA homeostasis and secondary mitochondrial dysfunction can have tragic consequences for patients with the occurrence of liver failure and severe lactic acidosis, which can eventually lead to death. Although this mechanism of mitochondrial toxicity might be rarer than direct inhibition of key mitochondrial enzymes involved in OXPHOS, TCA or mtFAO, it might actually be underestimated. Indeed, preclinical and post-marketing safety studies do not classically include specific investigations in order to detect drug-

induced effects on mtDNA integrity and maintenance. Different reasons could explain this fact. For instance, investigations looking for impairment of mtDNA translation require particular assays or tools such as an *in vitro* mitochondrial protein synthesis assay (McKee et al., 2006; Nagiec et al., 2005), or antibodies specifically targeting mtDNA-encoded polypeptides (Le Guillou et al., 2018; Will and Dykens, 2014). Moreover, *in vitro* or *in vivo* detection of drug-induced alteration of mtDNA homeostasis and its functional consequences can require several days (or weeks) of treatment (Larosche et al., 2007; Le Guillou et al., 2018; Mansouri et al., 2003; Will and Dykens, 2014). Hence, rapid and high-throughput assays cannot detect such mechanism of mitochondrial toxicity. Thus, efforts should be made in the future in order to look more often for mtDNA homeostasis impairment, in particular during preclinical safety studies. Hopefully, these endeavors might improve the safety profile of drugs that will enter clinical trials in the future. Finally, it should be underlined that investigations on mtDNA homeostasis cannot be restricted to antiviral drugs and antibiotics. Indeed, other pharmacological classes could be involved, as illustrated for example by studies on tacrine, tamoxifen and imipramine (Larosche et al., 2007; Le Guillou et al., 2018; Mansouri et al., 2003).

### **Conflict of interest disclosure**

The author declares that there is no conflict of interest regarding this work.

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## Legend to Figures

**Figure 1. Deleterious consequences of impaired mitochondrial DNA homeostasis.** Normal mitochondrial DNA (mtDNA) homeostasis is essential for the preservation of mitochondrial function in healthy liver. Indeed, mtDNA encodes 13 polypeptides involved in oxidative phosphorylation (OXPHOS) and ATP synthesis. This is associated with normal activity of mitochondrial fatty acid oxidation (mtFAO) and tricarboxylic acid (TCA) cycle (also referred to as Krebs cycle). Low levels of reactive oxygen species (ROS) are generated by the mitochondrial respiratory chain (MRC) in this physiological state. In contrast, a significant impairment of mtDNA homeostasis can reduce ATP synthesis and the activity of different metabolic pathways including mtFAO and TCA cycle. In addition, alteration of the MRC activity leads to ROS overproduction. These events can eventually induce serious liver injury with different types of hepatic lesions such as necrosis, steatosis (microvesicular and/or macrovacuolar), steatohepatitis and cirrhosis.

**Figure 2. Drugs inducing impairment of mitochondrial DNA homeostasis and involved mechanisms.** Different drugs have been reported to impair mitochondrial DNA (mtDNA) homeostasis by distinct mechanisms. Some drugs can inhibit mtDNA replication, thus leading to reduced mtDNA levels (also referred to as mtDNA depletion). In addition, mtDNA depletion can also be induced by an overproduction of reactive oxygen species (ROS) that oxidatively damage mtDNA, which in turn is completely degraded by several mitochondrial nucleases. Whatever its mechanism, mtDNA depletion is associated with lower levels of mtDNA-encoded rRNAs, tRNAs and mRNAs, thus reducing the synthesis of 13 polypeptides involved in OXPHOS. Other drugs can impair mtDNA translation via

their interaction with mitochondrial ribosomes, thus lowering the synthesis of these 13 OXPHOS proteins. Other mechanisms of mtDNA homeostasis impairment might include alteration of mitochondrial biogenesis and mtDNA methylation status, albeit further investigations are needed to determine their potential involvement in drug-induced liver injury. Other abbreviations used in this figure: APAP, acetaminophen (paracetamol); HSVtk, herpes simplex virus thymidine kinase; NRTIs, nucleoside reverse-transcriptase inhibitors; d4T, stavudine; ddI, didanosine; AZT, zidovudine.

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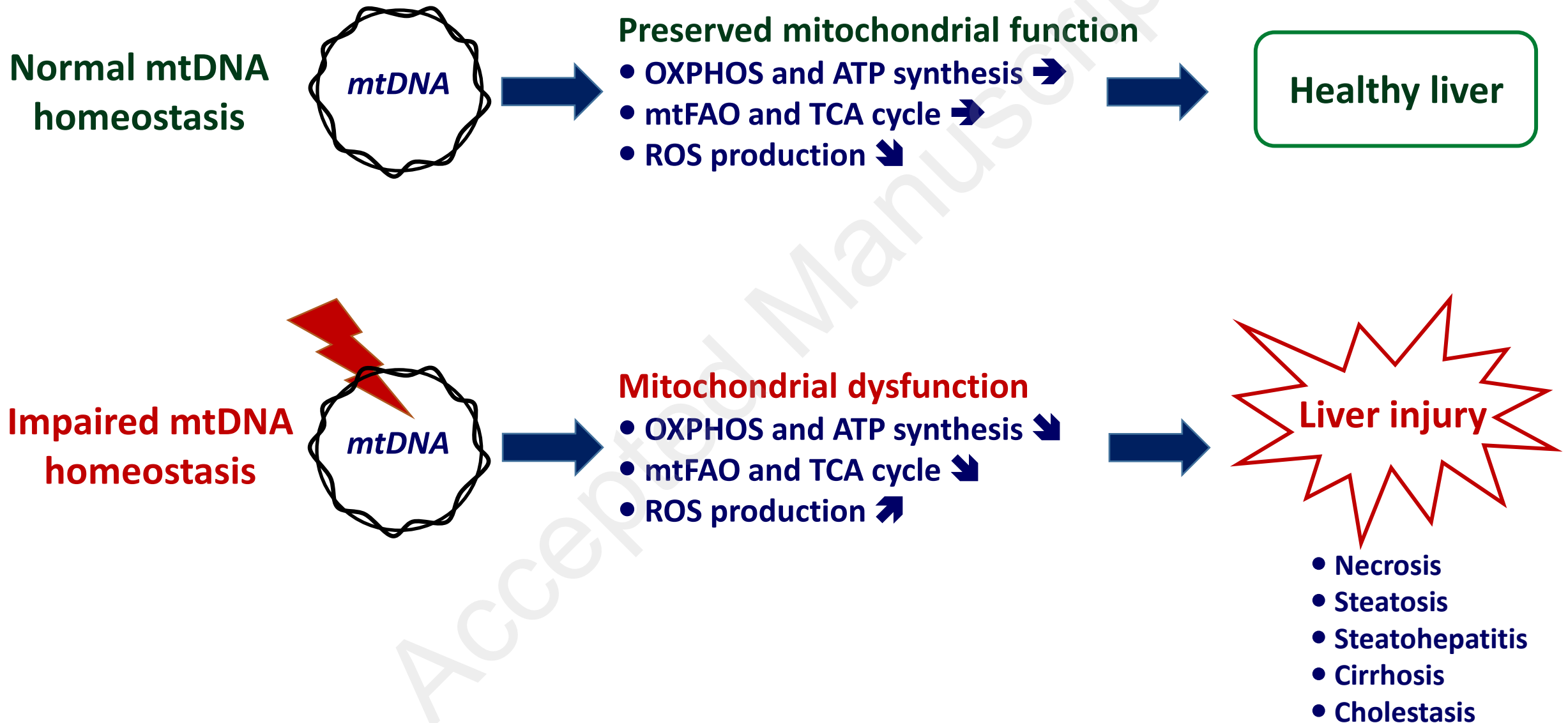


Figure 1



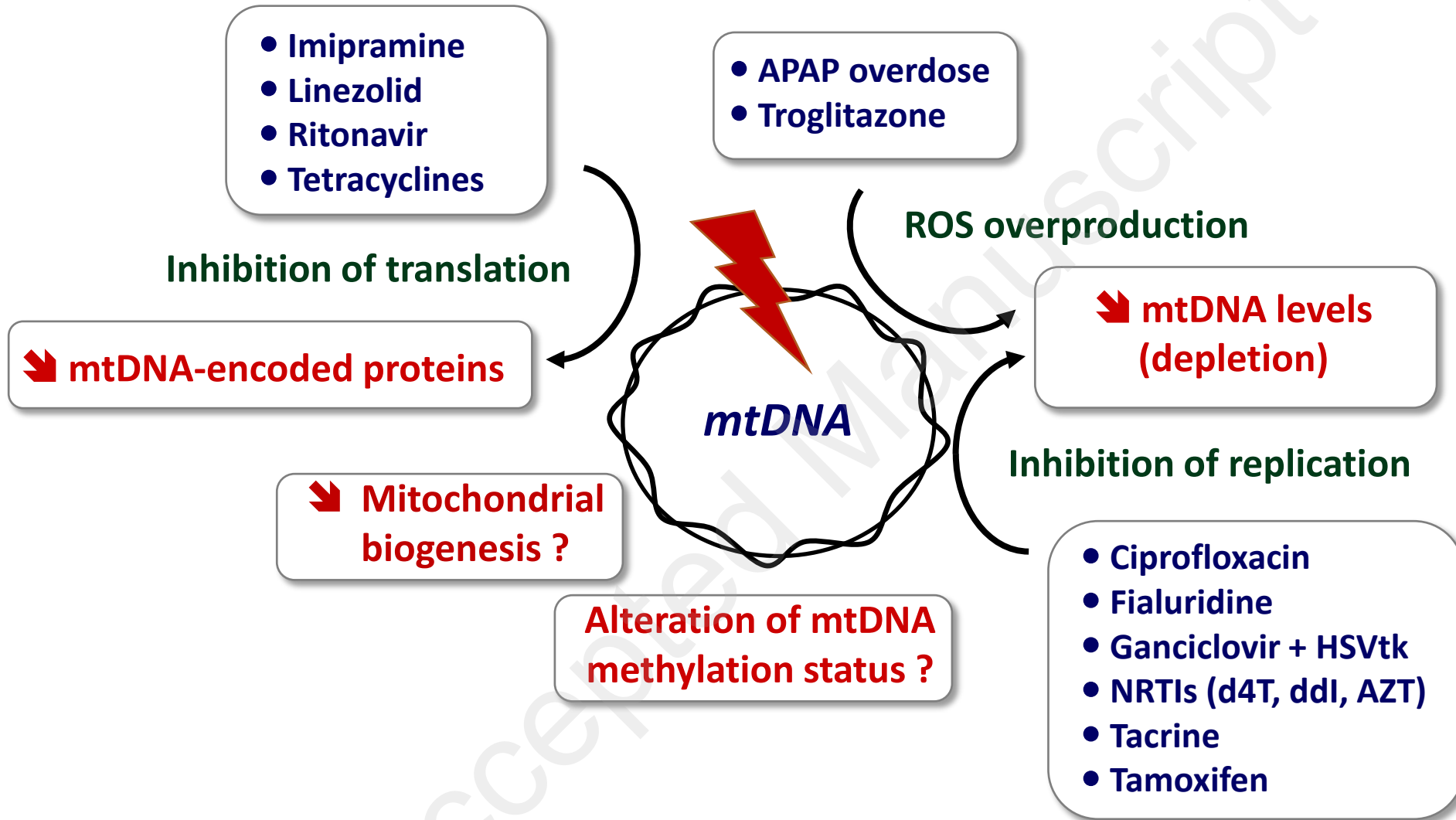


Figure 2