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Review Article

Inhibition of mitochondrial fatty acid oxidation in drug-induced hepatic steatosis☆

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ABSTRACT

Mitochondrial fatty acid oxidation (mtFAO) is a key metabolic pathway required for energy production in the liver, in particular during periods of fasting. One major consequence of drug-induced impairment of mtFAO is hepatic steatosis, which is characterized by an accumulation of triglycerides and other lipid species, such as acyl-carnitines. Actually, the severity of this liver lesion is dependent on the residual mitochondrial β-oxidation flux. Indeed, a severe inhibition of mtFAO leads to microvesicular steatosis, hypoglycemia and liver failure. In contrast, moderate impairment of mtFAO can cause macrovacuolar steatosis, which is a benign lesion in the short term. Because some drugs can induce both microvesicular and macrovacuolar steatosis, it is surmised that severe mitochondrial dysfunction could be favored in some patients by non-genetic factors (*e.g.*, high doses and polymedication), or genetic predispositions involving genes that encode proteins playing directly or indirectly a role in the mtFAO pathway. Example of drugs inducing steatosis include acetaminophen (APAP), amiodarone, ibuprofen, linezolid, nucleoside reverse transcriptase inhibitors, such as stavudine and didanosine, perhexiline, tamoxifen, tetracyclines, troglitazone and valproic acid. Because several previous articles reviewed in depth the mechanism(s) whereby most of these drugs are able to inhibit mtFAO and induce steatosis, the present review is rather focused on APAP, linezolid and troglitazone. These steatogenic drugs are indeed rarely discussed in the literature as regards their ability to impair mtFAO.

1. Introduction

At least 350 drugs of the modern pharmacopoeia have been reported to induce liver injury.[1,2] In the most severe cases, drug-induced liver injury (DILI) can require liver transplantation, or lead to the death of the patient.[2] Moreover, DILI can lead to the withdrawal of drugs from the market, or earlier during clinical trials, thus causing important financial losses.[3] Although not discussed in this review, it is noteworthy that many compounds including ethanol, herbal medicines, dietary supplements, and industrial chemicals can also induce liver injury.[4–6]

Pharmaceuticals are able to induce several types of liver injury, such as acute and chronic hepatitis, cholestasis, ductopenia, phospholipidosis and steatosis.[7,8] The latter liver lesion corresponds to hepatic accretion of lipids, mainly triglycerides, albeit other lipids species can also accumulate. Although hepatic steatosis seems to be a frequent lesion induced by drugs, its exact prevalence is currently unknown. Biour and collaborators[7] reported that about 12% of the hepatotoxic drugs could induce steatosis. However, this figure might be underestimated because steatosis does not always have any biological or clinical features. Indeed, whereas microvesicular steatosis is a severe liver lesion, macrovacuolar steatosis is deemed to be a benign lesion, at least in the short term.[9–11]

Numerous investigations reported that the impairment of mitochondrial fatty acid oxidation (mtFAO) is an important mechanism leading to drug-induced hepatic steatosis, although other metabolic pathways could be involved (see Section 4.2.4).[9,10,12] Notably, drugs can impair mtFAO by direct or indirect mechanisms, as discussed later on. In this review, I first recall the main features of mtFAO and the key regulators of this major metabolic pathway. Then, the main consequences of mtFAO impairment are presented, in particular concerning the accumulation of hepatic triglycerides and other lipid species. Next, drug-induced microvesicular and macrovacuolar steatosis are discussed in separate sections because these lesions present different clinicopathologic

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and biochemical features. Finally, I provide information regarding drugs able to induce steatosis by impairing mtFAO through different mechanisms. Actually, several previous articles reviewed in depth the mechanism(s) whereby different drugs are able to inhibit mtFAO and induce steatosis, or steatohepatitis.[9,10,13–18] In order to avoid major redundancies with these reviews, I have selected three steatogenic drugs, namely acetaminophen (APAP), linezolid and troglitazone, which are rarely discussed in the literature for their ability to impair mtFAO.

2. Main features of mtFAO and its regulation

2.1. The mtFAO pathway

The mtFAO pathway is a key metabolic process mandatory for the preservation of normal energy output, especially during periods of fasting. Readers are invited to peruse recent excellent reviews for detailed information regarding mtFAO.[19–22] Only the key features of this metabolic pathway will be mentioned below.

In contrast to the peroxisomal FAO, which primarily degrades very long-chain fatty acids (VLCFAs) with more than 22 carbons (C22), mtFAO is able to oxidize fatty acids (FAs) of different lengths including VLCFAs (>C20), long-chain FAs (LCFAs, C14 to C20), medium-chain FAs (MCFAs, C8 to C12) and short-chain FAs (SCFAs, <C6). Whereas SCFAs and MCFAs freely enter mitochondria, the mitochondrial entry of LCFAs and VLCFAs requires a specific shuttle system involving four different steps (Fig. 1). First, these FAs are activated into acyl-CoA thioesters by long-chain acyl-CoA synthetase (ACS), located in the outer mitochondrial membrane (OMM). Next, long-chain acyl-CoA thioesters are converted into acylcarnitines by carnitine palmitoyltransferase-1 (CPT1), which is also located in the OMM. Notably, CPT1 plays a major role in the regulation of LCFA mitochondrial oxidation during the feeding and fasting periods, as mentioned afterwards. The acyl-carnitines are then translocated across the inner mitochondrial membrane (IMM) into the mitochondrial matrix by carnitine-acylcarnitine translocase (CACT). Finally, carnitine palmitoyltransferase-2 (CPT2), located on the inner side of the IMM, transfers the acyl moiety from carnitine back to coenzyme A.

Once within mitochondria, all FAs undergo the β-oxidation process *per se*, which consists of four sequential reactions leading to the release of one acetyl-CoA molecule and an FA shortened by two carbons (Fig. 1). This shortened FA can be further oxidized by other cycles of mitochondrial β-oxidation (Fig. 1). Hence, in physiological conditions, endogenous FAs are entirely oxidized in acetyl-CoA molecules (for instance 8 molecules for palmitic acid). Importantly, two reactions of the mitochondrial β-oxidation process are catalyzed by different dehydrogenases using oxidized nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) as cofactors and having specific activities for SCFAs, MCFAs, LCFAs and VLCFAs, respectively. For instance, there are 4 different FAD-dependent dehydrogenases including short-chain acyl-CoA dehydrogenase (SCAD), medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCAD) and very long-chain acyl-CoA dehydrogenase (VLCAD). In the liver, a significant part of the mtFAO-derived acetyl-CoA molecules generate the ketone bodies (KBs) acetoacetate and β-hydroxybutyrate, especially during fasting (Fig. 1). KBs are subsequently released in the blood to be oxidized in extrahepatic tissues by the tricarboxylic acid (TCA) cycle.

2.2. Main factors regulating the mtFAO pathway

The physiological regulation of mtFAO is complex and involves numerous factors.[20,23,24] For instance, the expression of different enzymes involved in mtFAO and ketogenesis are positively regulated at the transcriptional level by peroxisome proliferator-activated receptor (PPAR) α, a nuclear receptor and transcription factor which can be stimulated by different natural FAs, or synthetic drugs such as fibrates.[5,20,24] In addition to PPARα, other transcription factors positively regulate hepatic mtFAO include PPARβ/δ, forkhead box A2 (FoxA2), cAMP-response element-binding protein (CREB) and hepatocyte nuclear factor-4α (HNF4α).[10,13] Moreover, both PPARγ coactivator-1α (PGC-1α) and PPARγ coactivator-1β (PGC-1β) play a key role in the transcriptional regulation of mtFAO enzymes.[10,16,20] Notably, mtFAO of LCFAs can be inhibited after a meal by malonyl-CoA, since this intermediate of *de novo* lipogenesis strongly inhibits CPT1. Hence, because of the fluctuating levels of hepatic malonyl-CoA depending on the nutritional status, mtFAO of LCFAs is stimulated during fasting and inhibited after feeding.[10,20,23] In pathophysiological conditions, other negative regulations can exist. For instance, any significant reduction in coenzyme A and L-carnitine levels can curb mtFAO.[9,25] A strong reduction of the mitochondrial respiratory chain (MRC) activity can also impair mtFAO. Indeed, inhibition of MRC activity limits the oxidation of reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) into NAD^+ and FAD, which are mandatory cofactors for the different dehydrogenases of the mitochondrial β-oxidation process.[9,10,26]

3. Main consequences of the impairment of mtFAO in liver

Whatever its origin, severe inhibition of mtFAO in the liver can induce adenosine triphosphate (ATP) shortage and cell death. Hence, mtFAO impairment can induce hepatic cytolysis, thus leading to increased levels of circulating alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[9,27] Actually, the impairment of hepatic mtFAO can not only induce energy deficiency in liver but also in extrahepatic tissues, thus leading to multiorgan failure.[9,28] This is because hypoketonemia impairs ATP generation in many tissues via reduced oxidation of KBs by the TCA cycle.

Inhibition of mtFAO can also impair hepatic gluconeogenesis, thus leading to hypoglycemia. Reduction in acetyl-CoA levels indeed impairs the activity of pyruvate carboxylase, a key regulatory enzyme in the gluconeogenesis pathway.[9,13,29] In addition, hypoglycemia could also be due to the general loss of hepatocytes and to higher extrahepatic utilization of glucose.[10,13] Importantly, hypoglycemia is expected to reinforce hypoketonemia-related ATP depletion and could favor brain damage.[9,29] Finally, when mtFAO impairment is secondary to MRC deficiency, hyperlactatemia and lactic acidosis can occur because the conversion of unmetabolized pyruvate to lactate by lactate dehydrogenase is favored by NADH accumulation.[26,30,31]

Inhibition of mtFAO can also induce the accumulation of intracytoplasmic lipids, mainly triglycerides but also other lipid species as mentioned below.[9,27] Whereas severe inhibition of mtFAO mainly induces microvesicular steatosis, moderate inhibition could be more often associated to macrovacuolar steatosis (Fig. 2), as discussed in Section 4.[9,10,13]

Although impairment of mtFAO mainly induces triglyceride deposition, other lipid derivatives can also accumulate, such as free fatty acids (FFAs), dicarboxylic acids, acyl-CoA thioesters, acylcarnitines and acylglycines.[3,9,10,27,28] The generation of carnitine and glycine conjugates is deemed to reduce the toxicity of FFAs and other deleterious derivatives such as dicarboxylic acids. Indeed, FFAs and dicarboxylic acids are toxic for mitochondria via different mechanisms, which may worsen cell dysfunction.[9,10,19] For instance, these lipid derivatives can uncouple oxidative phosphorylation (OXPHOS), inhibit MRC activity and induce

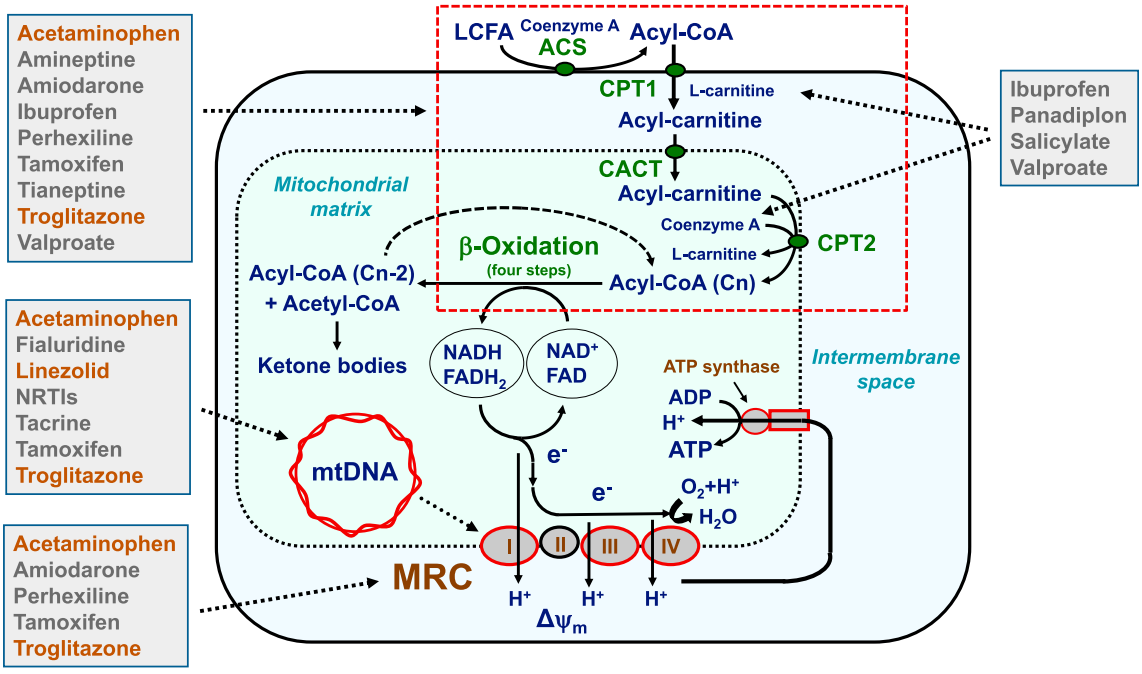

Fig. 1. mtFAO and main targets of different drugs are able to induce hepatic steatosis. Whereas short-chain and medium-chain fatty acids freely enter mitochondria (not shown), the entry of LCFAs requires a specific shuttle system involving four steps: (i) LCFAs are activated into acyl-CoA thioesters by long-chain ACS, located in the OMM. (ii) The long-chain acyl-CoA thioesters are converted into acyl-carnitines by CPT1, also located in the OMM. (iii) The acyl-carnitines are translocated across the IMM into the mitochondrial matrix by CACT. (iv) Finally, CPT2, located on the inner side of the IMM, transfers the acyl moiety from carnitine back to coenzyme A. Next, whatever their chain length, acyl-CoA thioesters (Cn) are oxidized via the β-oxidation process into fatty acyl-CoA thioesters shortened by 2 carbons (Cn-2) and an acetyl-CoA molecule. Whereas the Cn-2 fatty acyl-CoA thioesters re-enter a new β-oxidation cycle (*i.e.*, four steps), acetyl-CoA moieties can generate ketone bodies (mainly acetoacetate and β-hydroxybutyrate), which are released into the blood and used by extrahepatic tissues for energy production. The β-oxidation process generates reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$), which transfer their electrons (e^-) to the mitochondrial respiratory chain (MRC), thus regenerating oxidized nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) used for other β-oxidation cycles. Within the MRC, electrons are sequentially transferred to different polypeptide complexes (numbered from I to IV) embedded within the IMM. The final transfer of the electrons to oxygen takes place at the level of complex IV (*i.e.*, cytochrome c oxidase). Mitochondrial DNA (mtDNA) encodes 13 polypeptides that are components of complexes I, III, IV and V (*i.e.*, adenosine triphosphate (ATP) synthase). The flow of electrons within the MRC is coupled with the extrusion of protons (H^+) from the mitochondrial matrix to the intermembrane space, which creates the mitochondrial transmembrane potential, $\Delta\psi_m$. When ATP is needed, these protons re-enter the matrix through ATP synthase thus liberating part of the $\Delta\psi_m$ energy, which is used to phosphorylate ADP into ATP. Drugs can impair mtFAO through different mechanisms (dotted arrows): (i) Direct inhibition of one or several enzyme(s) involved in the whole mtFAO pathway (red dotted-line box), including ACS and CPT1 as well as enzymes catalyzing the four steps of the β-oxidation process *per se*. (ii) Sequestration of the mtFAO cofactors, L-carnitine and coenzyme A. (iii) Direct inhibition of MRC activity. (iv) Impairment of mtDNA homeostasis including replication and translation. The latter mechanism can eventually induce mtFAO impairment via an alteration of MRC activity. Drugs mentioned in this figure are only examples illustrating these different mechanisms. Drugs in brown letters namely APAP, linezolid and troglitazone are discussed in depth in this review. More details on mtFAO, MRC and mtDNA can be found in different former and recent reviews.[9,19–23,26,50] Additional information regarding other drugs impairing mtFAO can be found in other reviews.[9,10,13–18] Abbreviations: mtFAO, mitochondrial fatty acid oxidation; LCFAs, long-chain fatty acids; ACS, acyl-CoA synthetase; OMM, outer mitochondrial membrane; CPT1, carnitine palmitoyltransferase-1; IMM, inner mitochondrial membrane; CACT, carnitine-acylcarnitine translocase; CPT2, carnitine palmitoyltransferase-2; APAP, acetaminophen.

mitochondrial permeability transition (MPT) pore opening.[9,19] Notably, the profile of accumulation of acylcarnitines and other fatty acid species can give valuable information regarding the exact level of mtFAO impairment, in particular in the context of mitochondrial genetic disorders. For instance, the accumulation of octanoylcarnitine (C8) is pathognomonic of MCAD deficiency,[32] while increased C14–C18 acylcarnitines are observed in VLCAD deficiency.[33] However, it should be mentioned that acylcarnitine measurement cannot be performed in routine clinical practice since it requires complex and expensive analytical methods, such as tandem mass spectrometry (MS/MS).[34]

4. Drug-induced hepatic steatosis

Drugs can induce either microvesicular steatosis (sometimes referred to as microsteatosis) or macrovacuolar steatosis (occasionally referred to as macrovesicular steatosis), and sometimes both kinds of lipid deposition. Whereas microvesicular steatosis is rare as a pure clinicopathologic entity, macrovacuolar steatosis is the most common form of lipid accumulation induced by drugs and other xenobiotics.[9,12,18] It is still unknown why lipids accumulate preferentially as small and large droplets within the cytoplasm, respectively, in microvesicular and macrovacuolar steatosis. It has been hypothesized that the small size of the lipid droplets might be due to an "emulsification" of triglycerides by FFAs,[9] but this hypothesis has never been confirmed. Alternatively, the nature and/or the abundance of some proteins and enzymes wrapping the lipids could play a role.[10] Indeed, proteins such as perilipin (PLIN1), adipophilin (PLIN2), patatin-like phospholipase domain containing 2 (PNPLA2, also known as adipose triglyceride lipase) and cell death-inducing DFF45-like effector (CIDE) proteins play a major role in the growth and maturation of lipid droplets in the liver.[35,36] Finally, energy shortage secondary to severe mtFAO impairment might curtail the growth of lipid droplets.

4.1. Drug-induced microvesicular steatosis

4.1.1. Clinicopathologic and biochemical features

Drug-induced microvesicular steatosis is a rare but potentially severe liver lesion that can be associated with liver failure, encephalopathy and coma, thus leading to the death of some patients.[9–12,37] Patients may present with nonspecific symptoms, such as fatigue, nausea, vomiting, abdominal pain, hepatomegaly and jaundice.[9,37] Liver pathology classically shows the presence

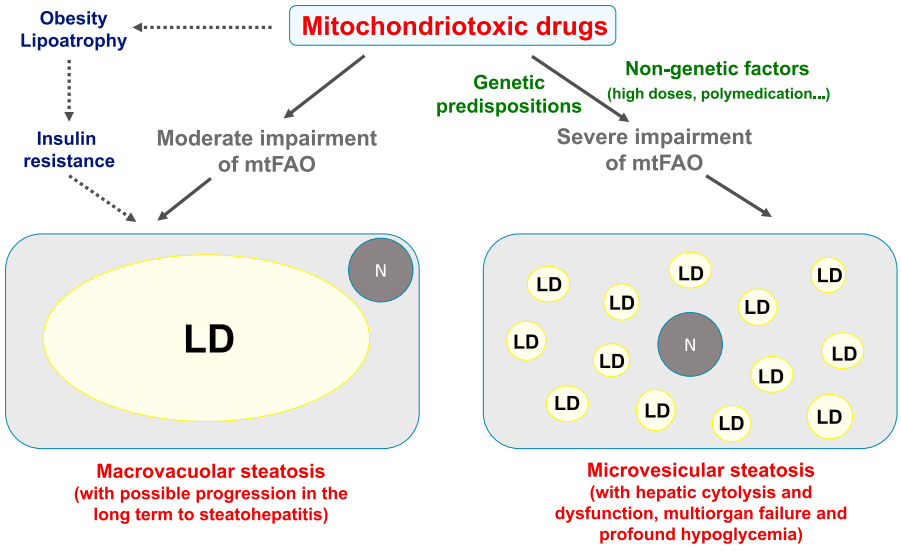

Fig. 2. Impairment of mtFAO and hepatic steatosis. The following mechanisms are currently put forward in order to explain why some mitochondriotoxic drugs can induce both macrovacuolar and microvesicular steatosis. Whereas a moderate impairment of mtFAO can explain the occurrence of macrovacuolar steatosis, a severe alteration of this metabolic pathway can induce microvesicular steatosis. Some mitochondriotoxic drugs such as valproic acid and the nucleoside reverse transcriptase inhibitors (NRTIs) stavudine and didanosine can also induce macrovacuolar steatosis by favoring adipose tissue dysfunction (*i.e.*, obesity or lipoatrophy) and related insulin resistance. In macrovacuolar steatosis, a large lipid droplet (LD) displaces the nucleus (N) at the periphery of the hepatocyte. In microvesicular steatosis, numerous small lipid droplets leave the N at the center of the hepatocyte. Although several hypotheses have been proposed (see Section 4), it is still unknown why lipids (mainly triglycerides) accumulate preferentially as small and large vacuoles within the cytoplasm, respectively, in microvesicular and macrovacuolar steatosis. Whatever the type of steatosis, triglyceride accumulation could be an adaptive protection against the cytotoxicity induced by some lipid derivatives, while the lipid droplets have no pathogenic role *per se*. Besides the involvement of non-genetic factors (*e.g.*, high doses and polymedication), severe impairment of mtFAO could be favored by the presence of genetic predispositions, such as congenital defects in enzymes involved in mtFAO and oxidative phosphorylation. In contrast to macrovacuolar steatosis, which is a benign lesion in the short term, microvesicular steatosis is a severe (and sometimes fatal) liver disease associated with liver dysfunction, hepatic cytolysis, multiorgan failure and profound hypoglycemia. Abbreviation: mtFAO, mitochondrial fatty acid oxidation.

within the cytoplasm of numerous lipid droplets, which usually leave the nucleus at the center of the hepatocyte.[8,11,18,37,38] However, isolated microvesicular steatosis is rare and is often associated with some macrovacuolar steatosis.[9,13,18] Regarding biochemical parameters, increased levels of serum ALT and AST can also be observed, thus reflecting hepatic cytolysis.[9,10,37] In addition, patients can present severe hypoglycemia, hyperammonemia, lactic acidosis, abnormal levels of plasma KBs, and accumulation of acylcarnitine derivatives and dicarboxylic acids in plasma and urine.[3,9,13,37] The latter abnormalities are related to the impairment of mtFAO, as previously discussed in Section 3.

4.1.2. *Drugs inducing microvesicular steatosis*

Drugs reported to induce microvesicular steatosis in patients are presented in Table 1.[9,10,12,18,37–48] Several remarks should be done regarding some of these drugs: (i) Because of their hepatotoxicity, fialuridine and panadiplon were withdrawn during clinical trials, whereas amineptine, troglitazone, perhexiline and pirprofen were withdrawn after marketing.[3,13,18] (ii) Drugs such as amiodarone and perhexiline induce more frequently macrovacuolar steatosis and steatohepatitis,[9,10,13] as mentioned later on. (iii) For some drugs including APAP,[40,41] L-asparaginase,[42] erythromycin,[38] ethinylestradiol,[43] imipramine,[44] indomethacin,[45] isoflurane,[46] linezolid[47] and niacin,[48] microvesicular steatosis has been reported in one patient, or a few patients at the most. Other reports should be needed in order to confirm the bona fide ability of these drugs to induce this hepatic lesion. Indeed, many other factors can bring about microvesicular steatosis, such as alcohol abuse, some toxins and different inborn errors of mitochondrial metabolism.[9,19,49,50]

4.1.3. *Mitochondrial dysfunction and drug-induced microvesicular steatosis*

It is now acknowledged that drug-induced microvesicular steatosis is mainly the consequence of a severe and prolonged inhibition of mtFAO (Fig. 2), whatever its mechanism.[3,9–13,18] Notably, a profound inhibition of mtFAO is also involved in the pathophysiology of microvesicular steatosis observed in other diseases, such as inborn errors of mtFAO, some mitochondrial

Table 1
Examples of drugs reported to induce microvesicular steatosis in patients.

Drugs	Therapeutic classes
Acetaminophen (APAP)	Analgesic, antipyretic
Amineptine	Antidepressant
Amiodarone	Antianginal, antiarrhythmic
Aspirin (and salicylic acid)[1]	NSAID
Didanosine (ddI)	Antiretroviral (anti-HIV)
Enalapril	Antihypertensive
Erythromycin	Antibiotic
Ethinylestradiol	Estrogenic hormone
Fialuridine (FIAU)	Antiviral (anti-HBV)
Ibuprofen	NSAID
Imipramine	Antidepressant
Indinavir	Antiretrovial (anti-HIV)
Indomethacin	NSAID
Isoflurane	General anesthetic
Ketoprofen	NSAID
L-asparaginase	Antineoplastic
Linezolid	Antibiotic
Niacin	Lipid-lowering agent
Panadiplon	Anxiolytic
Perhexiline	Antianginal
Pirprofen	NSAID
Stavudine (d4T)	Antiretroviral (anti-HIV)
Tetracycline and its derivatives (high doses)	Antibiotics
Tianeptine	Antidepressant
Troglitazone	Antidiabetic
Valproic acid	Antiepileptic
Zidovudine (AZT)	Antiretroviral (anti-HIV)

[1] Drugs in bold were reported to induce more frequently microvesicular steatosis than the others. Abbreviations: HBV, hepatitis B virus; HIV, human immunodeficiency virus; NSAID, nonsteroidal anti-inflammatory drug.

cytopathies and acute fatty liver of pregnancy.[9,19,49,50] All these severe liver diseases characterized by profound mitochondrial dysfunction and microvesicular steatosis are sometimes referred to as Reye-like syndromes. Indeed, their clinical course and hepatic histopathology are similar to Reye's syndrome, an illness mainly occurring in children and clearly related to mitochondrial dysfunction induced by aspirin and different post-infectious factors.[9]

It should be underlined herein that the clinical severity of Reye-like syndromes is not directly due to the accumulation of small lipid droplets but rather to the dysfunctional mtFAO pathway. Indeed, profound impairment of mtFAO in the liver can have different deleterious consequences on cell homeostasis including ATP shortage, cell death and hepatic cytolysis,[9,13,27] as previously mentioned (see Section 3). Hence, whatever its origin, microvesicular steatosis only reflects the presence of a severe inhibition of mtFAO and has no pathogenic role *per se*.

Drugs can inhibit mtFAO by different mechanisms that are not mutually exclusive (Fig. 1)[3,9,10,12,13]: (i) Direct inhibition of one or several mtFAO enzymes, including CPT1. (ii) Sequestration of coenzyme A and/or L-carnitine, two cofactors mandatory for mtFAO. (iii) Impairment of PPARα activity. (iv) Direct inhibition of MRC. (v) Impairment of mitochondrial DNA (mtDNA) homeostasis, including replication and translation. In case of impairment of mtDNA replication, which can lead to mtDNA depletion, the number of mtDNA copies must fall below 20–40% of basal levels to induce the impairment of MRC and mtFAO.[13,30,50]

4.2. Drug-induced macrovacuolar steatosis

4.2.1. Clinicopathologic and biochemical features

With some drugs, liver triglycerides accumulate as a large (often single) lipid vacuole that displaces the nucleus at the periphery of the hepatocyte.[8,14,18] This lesion, commonly referred to as macrovacuolar steatosis, is often observed in overweight and obese persons as well as in alcoholic patients.[4,18,51] At this stage, this lesion can be asymptomatic with the only presence of hepatomegaly. Whatever its cause, macrovacuolar steatosis can sometimes progress in the long term to steatohepatitis, which is characterized by necroinflammation, hepatocyte ballooning and some fibrosis.[4,8,14,51] Plasma transaminases (ALT and AST) can be elevated in some patients.[52,53] With drugs such as the antiretroviral nucleoside reverse transcriptase inhibitors (NRTIs) stavudine and didanosine, amiodarone, perhexiline and methotrexate, steatohepatitis can further progress to cirrhosis.[10,11,15,52] In severe cases, hyperbilirubinemia, low albumin levels and prolonged prothrombin time can also be observed, thus reflecting liver failure.[52]

4.2.2. Drugs inducing macrovacuolar steatosis

Drugs reported to induce macrovacuolar steatosis (and sometimes steatohepatitis) in patients are presented in Table 2.[9,10,15,16,18,39,52–59] Several comments should be done concerning some of these drugs. (i) Drugs such as glucocorticoids, olanzapine, risperidone and valproate can induce macrovacuolar steatosis and steatohepatitis by promoting obesity and insulin resistance.[13,18,60,61] (ii) Conversely, other drugs including stavudine and other antiretroviral agents can favor hepatic fat accumulation via lipoatrophy (*i.e.*, a severe reduction of body fat mass), which can also promote insulin resistance.[13,18,26] (iii) For mitochondriotoxic drugs inducing obesity (*e.g.*, valproate) or lipoatrophy (*e.g.*, stavudine), it cannot be excluded that macrovesicular steatosis develops in patients also as a direct consequence of moderate mtFAO impairment in the liver (Fig. 2).[10,13,18]

Table 2
Examples of drugs reported to induce macrovacuolar steatosis in patients.

Drugs	Therapeutic classes
Allopurinol	Urate-lowering (antigout)
Amiodarone[1]	Antianginal, antiarrhythmic
Anabolic steroids	Androgenic hormones
Cisplatin	Antineoplastic
Carbamazepine	Antiepileptic
Chloroquine	Antimalarial
Didanosine (ddI)	Antiretroviral (anti-HIV)
Diltiazem	Antianginal, antiarrhythmic
D-penicillamine	Copper chelator
Estrogens	Estrogenic hormones
5-Fluorouracil	Antineoplastic
Glucocorticoids	Anti-inflammatory
Irinotecan	Antineoplastic
L-asparaginase	Antineoplastic
Lomitapide	Lipid-lowering
Methimazole	Antithyroid
Methotrexate	Antipsoriatic, anti-rheumatoid
Metoprolol	Antihypertensive
Mipomersen	Lipid-lowering
Nifedipine	Antianginal, antihypertensive
Olanzapine	Antipsychotic
Perhexiline	Antianginal
Raloxifene	SERM, anti-osteoporotic
Rifampicin	Antibiotic
Risperidone	Antipsychotic
Stavudine	Antiretroviral (anti-HIV)
Sulindac	NSAID
Tamoxifen	SERM, antineoplastic
Toremifene	SERM, antineoplastic
Valproic acid	Antiepileptic
Verapamil	Antiarrhythmic

[1] Drugs in bold were reported to induce steatohepatitis in some patients. Abbreviations: HIV, human immunodeficiency virus; NSAID, nonsteroidal anti-inflammatory drug; SERM, selective estrogen receptor modulator.

4.2.3. Mitochondrial dysfunction and drug-induced macrovacuolar steatosis

Mitochondrial dysfunction could be involved in the pathogenesis of macrovacuolar steatosis, at least for some drugs such as amiodarone, perhexiline, the NRTIs stavudine and didanosine. For these compounds, macrovacuolar steatosis might be associated with mild or moderate impairment of mtFAO.[10,13,18] Such mild-to-moderate mtFAO impairment is thought to favor triglyceride accumulation and to have no (or limited) deleterious consequences on ATP production and hepatocyte viability, thus explaining why levels of plasma transaminases can be in the normal range in drug-induced macrovacuolar steatosis. Importantly, and similarly to microvesicular steatosis, triglyceride accumulation as large vacuoles has no pathogenic role *per se*. In the context of liver injury induced by drugs and other xenobiotics, macrovacuolar steatosis only reflects an impairment of lipid metabolism, whatever the underlying mechanism(s) (see Section 4.2.4).[4,10,12–14]

It is noteworthy that some drugs inducing macrovacuolar steatosis (*e.g.* amiodarone, perhexiline, NRTIs) are able to impair both mtFAO and MRC activity.[9,13,15–17] For these drugs, it has been proposed that mild-to-moderate MRC impairment, might be involved in the pathogenesis of the transition from steatosis to steatohepatitis.[14–18,52] Indeed, by altering MRC activity, these drugs could favor reactive oxygen species (ROS) generation at the level of complexes I and III. Overproduction of ROS in a cellular environment enriched in fat can in turn trigger lipid peroxidation and the production of reactive aldehydes that have many deleterious effects on the liver.[13,62] For instance, mitochondrial ROS and lipid peroxidation-derived aldehydes have been shown to bring about cell death,[14,15,17] and to play a role in liver inflammation and fibrosis in different pathophysiological situations.[63,64] In steatohepatitis,

the presence of hepatic necroinflammation can induce a moderate increase in plasma transaminases.[52,53]

Genetic predispositions could also favor the pathogenesis of drug-induced steatohepatitis. Unfortunately, data regarding this important issue is scarce. Polymorphisms, or mutations, in the cytochrome P450 2D6 *(CYP2D6)* gene greatly increased the risk of perhexiline-induced steatohepatitis.[52] It would be interesting to determine whether the common patatin-like phospholipase domain containing 3 (PNPLA3) rs738409 genotype might favor drug-induced steatohepatitis. This genetic variant was indeed reported to significantly favor the occurrence of this lesion in the context of alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), a large spectrum of liver lesions linked to obesity.[65] Importantly, beyond its role in triglyceride metabolism, PNPLA3 could also regulate mitochondrial function.[66] Finally, superoxyde dismutase 2 (SOD2) polymorphism could also be interesting to study in the context of drug-induced steatohepatitis, similarly to non-alcoholic steatohepatitis (NASH).[67,68]

4.2.4. Involvement of non-mitochondrial pathways in drug-induced macrovacuolar steatosis

Besides mitochondrial function, some drugs could induce macrovacuolar steatosis by impairing the secretion of very-low-density lipoprotein (VLDL),[13,18,58,69,70] although the related mechanism is still unknown for most of these drugs. Some drugs, such as amiodarone, are able to inhibit the activity of microsomal triglyceride transfer protein (MTP), an enzyme that lipidates apolipoprotein B to form VLDL.[71] A role of endoplasmic reticulum (ER) stress could also be an attractive hypothesis given the major negative impact of this stress on VLDL secretion.[72] Alternatively, drugs can favor the accumulation of fat by activating *de novo* lipogenesis. This effect can be secondary to a direct activation of lipogenic transcription factors, such as sterol regulatory element binding protein 1c (SREBP1c) and PPARγ, or be indirect via drug-induced obesity (or lipoatrophy) and related hyperinsulinemia.[13,61,73] Finally, impairment of autophagy might also be involved in drug-induced macrovacuolar steatosis, as recently proposed for irinotecan.[74]

4.3. Drugs inducing microvesicular and macrovacuolar steatosis

Different drugs such as amiodarone, perhexiline and NRTIs (*e.g.*, stavudine and didanosine) can induce both microvesicular and macrovacuolar steatosis (Tables 1 and 2). Actually, it is still unclear why some mitochondriotoxic drugs can induce microvesicular steatosis in a few patients, while they induce more frequently macrovacuolar steatosis in others. It is conceivable that genetic predispositions could play a role, such as congenital defects in enzymes involved in mtFAO, OXPHOS and antioxidant defense.[9,10,13] Indeed, these congenital defects are expected to favor drug-induced mitochondrial dysfunction in treated patients, thus leading to mitochondrial failure and microvesicular steatosis (Fig. 2). For instance, mutations (or polymorphisms) in genes encoding MCAD and DNA polymerase γ (POLG) seem to favor drug-induced severe mitochondrial dysfunction and microvesicular steatosis induced by some drugs, such as valproic acid and NRTIs.[10,13,75] Again, it might be of interest to search for genetic variations of SOD2 in patients. Interestingly, an SOD2 polymorphism in alcoholics significantly increased the risk of developing microvesicular steatosis, while it did not change that of macrovacuolar steatosis.[76] Non-genetic factors might also favor severe mitochondrial dysfunction and thus the occurrence of microvesicular steatosis. These factors were for instance polytherapy and young age for valproic acid and high doses for different tetracycline derivatives.[9]

5. Examples of drugs inducing mtFAO impairment and steatosis

As already mentioned, several previous articles reviewed the mechanism(s) whereby different drugs are able to impair mtFAO and induce steatosis, or steatohepatitis.[9,10,13–18] Examples of drugs extensively discussed in these articles include amiodarone, ibuprofen, NRTIs such as stavudine and didanosine, perhexiline, tamoxifen, tetracyclines and valproic acid. Hence, in order to avoid major repetitions with these reviews, I have selected other steatogenic drugs rarely discussed in the literature as regards their ability to impair mtFAO.

5.1. APAP

5.1.1. APAP-induced liver injury and steatosis

APAP, also known as paracetamol, is one of the most widely prescribed drugs for the management of pain and hyperthermia. The current maximum recommended dosage of APAP is 4000 mg/d in adults although the United States Food and Drug Administration (FDA) suggested a reduction of this dose to 3250 mg/d or 3000 mg/d, which was driven by a concern regarding the incidence of acute liver failure (ALF) due to APAP overdose.[77,78] Indeed, APAP intoxication after voluntary or unintentional overdoses can lead to massive hepatocellular (mostly centrilobular) necrosis and ALF requiring liver transplantation.[79,80] Interestingly, steatosis can be observed in residual viable hepatocytes.[8,40] Therapeutic doses of APAP can also induce mild to moderate hepatic cytolysis in a significant proportion of individuals,[81,82] and possibly fulminant hepatitis in a few patients.[83,84] Massive hepatic necrosis and microvesicular steatosis were recently reported in a child after repeated administration of therapeutic APAP doses.[41] Increased hepatic triglycerides and microvesicular steatosis have also been observed in rodents treated with low and high APAP doses.[85–90] Finally, fatty change and microvesicular steatosis were also reported in a dog model of APAP-induced fulminant hepatic failure.[91]

5.1.2. Mechanisms of APAP-induced liver necrosis

APAP is mainly metabolized in the liver into the nontoxic sulfate and glucuronide conjugates. However, a small amount of APAP is oxidized by cytochrome P450 2E1 (CYP2E1) and cytochrome P450 3A4 (CYP3A4) to the reactive metabolite N-acetyl-*p*-benzoquinone imine (NAPQI).[92,93] When APAP is taken at the recommended dosage, and in the absence of predisposing factors (*e.g.*, ALD, frailty, NAFLD …), NAPQI is usually detoxified by cytosolic and mitochondrial glutathione (GSH). In contrast, after APAP overdose, high levels of NAPQI can induce cell death. Indeed, once GSH is deeply depleted and no longer available for NAPQI detoxification, this reactive metabolite binds to different proteins, especially within the mitochondria.[93,94] This generation of APAP-protein adducts induces a profound and irreversible impairment of MRC and OXPHOS, thus leading to ATP shortage, massive hepatocellular necrosis and ALF.[80,95] Among the different components of the MRC, complex II (succinate dehydrogenase) seems to be very sensitive to the inhibitory effect of NAPQI.[96,97]

MRC impairment might also result from a significant depletion of hepatic mtDNA,[92,98,99] which is induced by the degradation of mtDNA copies harboring numerous oxidative lesions. This is because mitochondria contain several enzymes, including endonucleases and exonucleases, able to remove the mtDNA copies that are too damaged to be efficiently repaired.[100,101] Recent investigations also reported that APAP-induced mitochondrial dysfunction involved p53 up-regulated modulator of apoptosis (PUMA) in a c-Jun N-terminal kinase (JNK)-dependent manner.[102]

However, some studies did not support the pathogenic role of JNK activation in APAP-induced liver injury.[93,103]

Besides NAPQI-induced mitochondrial dysfunction, several studies reported that APAP can induce a direct and reversible inhibition of MRC activity, in particular at the level of complex III.[104,105] APAP might also impair the activity of the TCA cycle in a NAPQI-independent manner, possibly via a reduction of MRC activity.[106] However, further investigations would be needed in order to determine whether these direct effects on mitochondrial function play a significant role in APAP-induced liver injury.

5.1.3. APAP-induced impairment of mtFAO and steatosis

Different experimental investigations reported that APAP intoxication was associated with an accumulation of different acylcarnitines in serum and liver, thus reflecting an impairment of mtFAO in the remaining viable hepatocytes. The first study reporting serum acylcarnitine accumulation in mice after APAP intoxication (400 mg/kg) was published in 2009 by Frank Gonzalez's group.[107] In this study, four long-chain acylcarnitines were detected, namely myristoylcarnitine (C14), palmitoylcarnitine (C16), palmitoleoylcarnitine (C16:1) and oleoylcarnitine (C18:1). Because levels of serum acylcarnitines were significantly reduced in *Cyp2e1*-null mice,[107] it is conceivable that NAPQI might have played a role in their accumulation. In 2013, Laura James's team confirmed the accumulation of serum myristoylcarnitine, palmitoylcarnitine and oleoylcarnitine in B6C3F1 mice after APAP intoxication (200 mg/kg), consistent with an inhibition of mitochondrial LCFA oxidation even with this moderate dose of APAP.[108] Interestingly, acylcarnitine elevation was concomitant with the onset of intrahepatic lipid deposition as assessed with oil red O staining. In 2014, Hartmut Jaeschke and collaborators also reported the presence of serum acylcarnitine accumulation in C57Bl/6 mice after APAP intoxication (300 and 600 mg/kg).[109] In particular, serum palmitoylcarnitine, oleoylcarnitine (or a close analog) and linoleoylcarnitine (C18:2) were detected, again consistent with an inhibition of mitochondrial LCFA oxidation.[109] Interestingly, the authors showed in this study that 500 mg/kg furosemide (a drug also leading to centrilobular necrosis but without primarily affecting mitochondrial function) did not cause any significant elevations in serum acylcarnitine levels.[109] Two other studies in mice reported that APAP-induced elevation of serum transaminases (ALT and AST) and different long-chain acylcarnitines (including palmitoylcarnitine and oleoylcarnitine) could be prevented by a pretreatment with different plant ingredients.[110,111] However, the exact mechanism of this protective effect was not determined in the latter investigations. Lastly, one study reported increased levels of intrahepatic palmitoylcarnitine and oleoylcarnitine in B6C3F1 mice after APAP intoxication (200 mg/kg).[112]

Clinical investigations were also performed in order to detect serum acylcarnitines after APAP intoxication. Indeed, Laura James's team reported the presence of serum palmitoylcarnitine and oleoylcarnitine accumulation in children with APAP overdose, but myristoylcarnitine was not increased compared to the controls.[113] Interestingly, serum palmitoylcarnitine and oleoylcarnitine were also significantly enhanced in hospitalized children receiving APAP as part of standard care, although serum ALT activity was not significantly elevated in these patients.[113] Investigations were also performed by McGill *et al.*[109] in serum samples from APAP overdose patients, but none of the above-mentioned acylcarnitines were elevated. According to the authors, the lack of acylcarnitine accumulation is most probably due to the standard N-acetylcysteine treatment given to patients, a hypothesis that was supported by mouse experiments.[109]

In conclusion, the profile of APAP-induced acylcarnitine accumulation seems to reflect a specific impairment of mitochondrial LCFA oxidation, most probably in the liver. This mitochondrial effect could explain why APAP is able to induce microvesicular steatosis in rodents,[87–90] dog and human.[40,41,91] Although the aforementioned investigations did not determine the exact mechanisms whereby APAP can inhibit mitochondrial LCFA oxidation, it is conceivable that NAPQI covalently binds to (and thus inhibits) a mitochondrial enzyme involved in LCFA oxidation. An inhibition of CPT1 can be excluded because CPT1 impairment is associated with reduced long-chain acylcarnitine levels.[114,115] Alternatively, APAP-induced inhibition of MRC could indirectly impair mtFAO, as already mentioned (see Section 2.2). Indeed, while MRC defects can induce variable acylcarnitine profiles, specific accumulation of long-chain acylcarnitines has previously been reported.[115] Finally, experimental investigations also showed that APAP intoxication significantly reduced hepatic PPARα expression,[92,116] which might participate in the impairment of mtFAO. Stabilization of hypoxia-inducible factor-1α (HIF-1α) seems to be involved in the hepatic down-regulation of PPARα expression after APAP overdose.[116]

5.2. Linezolid

5.2.1. Linezolid-induced hepatic and extrahepatic toxicity

The oxazolidinone linezolid is an antibiotic particularly efficient against drug-resistant pathogens including the Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecium* as well as *Mycobacterium tuberculosis*.[117] Like many other antibiotics, linezolid impairs bacterial growth by inhibiting protein synthesis via its binding to bacterial ribosomes.[117] Moderate to severe 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.[47,118–120] Accumulation of lipid globules was also reported in patient myofibers.[121] In addition, severe and potentially lethal lactic acidosis has been reported in some patients.[47,117,122] Hence, blood lactate levels should be regularly monitored during prolonged linezolid therapy.[117] Hypoglycemia has also been described in patients, sometimes associated with lactic acidosis.[117,122,123] Prolonged administration of linezolid can also induce skeletal myopathy and rhabdomyolysis, pancreatitis, stroke-like episodes, peripheral and optic neuropathy, thrombocytopenia, bone marrow suppression and renal failure.[50,117,121,122,124] Notably, the spectrum of linezolid-induced adverse effects is similar to the clinical manifestations observed in patients with genetic defects of OXPHOS.[9,50,118] Long-term treatment, high doses and elevated linezolid blood concentrations could greatly enhance the risk of adverse effects induced by this antibiotic.[117,124] Genetic predispositions could also play a role, as discussed in the next section.

5.2.2. Linezolid-induced mitochondrial dysfunction

Linezolid is a potent inhibitor of mammalian mitochondrial protein synthesis via its interaction with the mitochondrial ribosomes.[117,125] Indeed, mammalian mitochondrial ribosomes have some similarities with bacterial ribosomes, in particular, at the level of the linezolid-binding site.[117,125] Inhibition of mtDNA translation secondarily reduces the activity of MRC complexes that contain mtDNA-encoded proteins, namely complexes I, III, IV and V.[117,119,126] It is now acknowledged that several linezolid-induced adverse effects including lactic acidosis, myelosuppression and neuropathy are related to the impairment of mtDNA translation.[50,117,127] Unfortunately, the relationship between the impairment of mtDNA translation in liver and steatosis is less documented, although some investigations in human and rat liver samples supported this assumption.[119] Interestingly, a previous study reported that linezolid impaired mtDNA translation in isolated rat liver mitochondria

with an IC_{50} of 12.8 ± 2.8 μM,[128] a concentration that is well below the mean serum C_{max} in patients (~45–55 μM).[129,130] Moreover, we also reported in differentiated human HepaRG cells that a 14-day treatment with 20 μM linezolid reduced the levels of two mtDNA-encoded polypeptides (*i.e.*, ND1 and cytochrome *c* oxidase (COX) 2), without any effect on the nuclear DNA-encoded COX4.[131] Importantly, 20 μM linezolid did not alter the protein expression of ND1 and COX2 in HepaRG cells treated for 3 days, thus indicating that prolonged linezolid exposure is needed in order to impair mtDNA translation in hepatic cells.[131] This is in contrast to other cell types such as human promyelocytes and monocytes for which a significant impairment of mtDNA translation was observed after 3 days of linezolid treatment.[130]

As already mentioned (see Section 3), any severe impairment of MRC activity can impair mtFAO. Although not directly demonstrated for linezolid, such relationship has been already shown (or highly suspected) for xenobiotics strongly inhibiting MRC activity, either directly (*e.g.*, rotenone and potassium cyanide),[132,133] or indirectly by reducing mtDNA levels (*e.g.*, NRTIs such as zidovudine and zalcitabine).[134,135] In addition, impairment of mtFAO has been reported in patients with congenital MRC defects.[28,115,136]

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.[117,121,127,137] However, further investigations will be needed to decipher the precise mechanism whereby these genetics might favor linezolid-induced mitochondrial dysfunction and to determine whether they might also increase the risk of liver injury and in particularly steatosis.

5.3. Troglitazone

5.3.1. Troglitazone-induced liver injury

The thiazolidinedione (TZD) troglitazone is the first PPARγ agonist used for the treatment of type 2 diabetes. TZD derivatives enhance insulin sensitivity by different mechanisms including the reduction of circulating FFAs, increased adiponectin secretion and the stimulation of energy expenditure.[138,139] These favorable effects are deemed to be primarily due to the direct action of TZD derivatives on white adipocytes, where PPARγ activation leads to increased lipid storage capacity and adiponectin synthesis.[139] In addition, TZD-induced PPARγ activation in brown-like adipocytes located in white adipose tissue may favor the stimulation of energy expenditure by enhancing mtFAO.[139] TZD derivatives could also directly improve insulin sensitivity in liver and skeletal muscle by activating AMP-activated protein kinase (AMPK).[138,139] Troglitazone has also been used in a pilot study for the treatment of NASH.[140] However, troglitazone has been withdrawn from the market in 2000 after the occurrence of several dozens of cases of severe (sometimes fatal) liver injury.[141,142] In most patients, the histopathologic changes included massive necrosis but other lesions such as cholestasis, steatosis, fibrosis and cirrhosis have also been reported.[7,143,144] Microvesicular steatosis in the residual hepatocytes, sometimes associated with macrovacuolar steatosis, has been reported in some patients with troglitazone-induced liver injury.[144–146] Hepatic steatosis has also been observed in rodents treated with troglitazone.[147,148]

5.3.2. Mechanisms of troglitazone-induced liver necrosis

Following troglitazone withdrawal from the market, numerous experimental investigations have been performed in order to determine the mechanism(s) whereby this TZD induced liver necrosis in a significant number of treated patients. Not surprisingly, troglitazone was found to be a potent mitochondriotoxic compound.[149,150] Indeed, troglitazone is able to significantly inhibit MRC activity and mitochondrial respiration for concentrations lower than 20 μM.[151,152] All the MRC complexes are inhibited by troglitazone although complex II seems to be less sensitive.[152,153] In addition, troglitazone could also indirectly impair MRC activity by inducing oxidative mtDNA damage such as strand breaks, which might have participated to the reduction of mtDNA levels in the treated cells.[152,154] Thus, this mechanism of mtDNA depletion seems similar to what has been proposed for APAP (Section 5.1.2) and other hepatotoxicants such as ethanol.[50,100]

Troglitazone was also shown to induce MPT pore opening in isolated rodent liver mitochondria in condition of calcium pulse.[155–157] However, troglitazone-induced mitochondrial membrane permeabilization could not be observed in mouse liver mitochondria in the absence of this calcium pulse.[151] Interestingly, investigations in the human hepatic cell line HC-O4 treated by troglitazone suggested that MPT pore opening was induced by ROS generated as a consequence of MRC inhibition.[158] Thus, although MPT pore opening appears to be an important mechanism whereby troglitazone induces cell death,[158,159] the above-mentioned data suggest that troglitazone might not be able to directly induce MPT pore opening in hepatocytes. Finally, troglitazone-induced mitochondrial impairment might be reinforced by a reduction of mitochondrial GSH levels, possibly due to lower GSH import into mitochondria.[160]

Troglitazone can be metabolized into several highly reactive metabolites by different hepatic CYPs including CYP3A4, CYP2C8 and CYP2C19.[141,142] In particular, these CYPs generate a quinone and a quinone epoxide derivatives.[141,161] Although these reactive metabolites have been postulated to be involved in troglitazone-induced hepatotoxicity,[161,162] investigations carried out in HepG2 cells and other data did not support a pathogenic role of these metabolites.[155,159,163] Notably, troglitazone has been reported to be a potent inducer of CYP3A4 and high levels of this enzyme might actually protect against troglitazone-induced cytotoxicity.[142,155,164] Hence, different aforementioned studies suggest that troglitazone-induced liver injury could be linked to the parent molecule rather than to CYP-generated reactive metabolites.

5.3.3. Troglitazone-induced impairment of mtFAO and steatosis

Despite numerous studies regarding troglitazone-induced mitochondrial dysfunction, only a few investigations focused on mtFAO. Reduced hepatic mtFAO and ketogenesis were reported in fructose-fed rats treated for 5 weeks with 70 mg/kg/d troglitazone.[165] A short-term treatment (*i.e.*, 1 h) with 100 μM or 1 mM troglitazone has been shown to impair the mitochondrial oxidation of oleic acid in isolated rat hepatocytes.[166] Moreover, the latter study disclosed that troglitazone was able to inhibit long-chain ACS, an effect expected to impair the mitochondrial entry of LCFAs (Fig. 1).[166] In contrast, by using human differentiated HepaRG cells, recent experiments in our laboratory showed that a 4-day treatment with 50 μM troglitazone did not inhibit mtFAO despite inducing significant ATP depletion and steatosis (unpublished results). Further investigations in rodent and human hepatocytes would be needed in order to explain the discrepancies of these results. Finally, troglitazone was reported to inhibit palmitate oxidation in isolated rat skeletal muscle.[167] Troglitazone-inhibition of mtFAO in skeletal muscle might have explained the occurrence of myositis and rhabdomyolysis in a few treated patients.[145,168] Skeletal muscle disorders including rhabdomyolysis can be observed in patients suffering from genetic disorders of the mtFAO pathway.[19,20,28]

5.3.4. Factors predisposing to troglitazone-induced liver injury

The idiosyncratic nature of liver toxicity associated with troglitazone treatment is supported by the apparent discrepancy between minimal hepatic effects observed in preclinical animal studies and the high hepatotoxic potential of troglitazone in human.[141,142,161] In addition, idiosyncrasy is reinforced by the fact that most of the deleterious effects induced *in vitro* by troglitazone were observed for concentrations well above those found in patients. Indeed, troglitazone most often induced significant toxicity for concentrations equal or superior to 10 μM,[152,154–159] whereas free troglitazone levels in patients were generally below this threshold concentration.[141,161] Hence, it has been proposed that the high hepatotoxic potential of troglitazone in human might be explained by different predisposing factors.[141,142,157,158,161]

First, the combined glutathione S-transferase GSTT1-GSTM1 null genotype and polymorphisms in the CYP2C19 gene might have played a role, at least in the Japanese population.[169,170] Second, obesity and related metabolic diseases such as type 2 diabetes and NAFLD might also have played a role.[161] In keeping with this assumption, troglitazone markedly induced microvesicular steatosis in diabetic KKA^{y} mice but not in lean control mice.[147] Moreover, a recent study showed that troglitazone (10–50 μM) induced MPT pore opening in liver mitochondria isolated from obese and diabetic Zucker diabetic fatty (ZDF) fa/fa rats whereas this mitochondriotoxic effect was not observed for the ZDF lean rats.[157] By using a cellular model of NAFLD induced by lipid overload, we also showed that troglitazone was significantly more cytotoxic in steatotic cells compared with nonsteatotic cells, as assessed by measuring cellular ATP levels.[131] Indeed, in human differentiated HepaRG cells treated for 2 weeks with troglitazone, the IC_{50} values for cytotoxicity were 70 and 59 μM in nonsteatotic and steatotic HepaRG cells, respectively.[131] Interestingly, our cellular model of NAFLD was associated with lower CYP3A4 activity,[131] similar to what has been observed in NAFLD patients.[73,171] It is also noteworthy that different experimental and clinical investigations reported that NAFLD can be associated with reduced MRC activity.[23,172,173] Taken together, these studies suggest that troglitazone-induced severe hepatotoxicity in obese patients with type 2 diabetes might have been favored by lower troglitazone biotransformation and preexisting reduced MRC activity, thus leading to greater mitochondrial dysfunction. Hepatic inflammation linked to NAFLD might also have played a role.[174]

6. Conclusions

Although it is now acknowledged that mtFAO inhibition could be commonly involved in drug-induced steatosis, and especially in microvesicular steatosis, the exact mechanisms of mtFAO impairment are still unknown for numerous steatogenic compounds. However, the determination of such mechanisms is a difficult task because a given drug can impair mtFAO by different direct and indirect pathways (Fig. 1). Nevertheless, high-throughput screening by using isolated rodent liver mitochondria can be useful in order to rapidly determine the potential deleterious effects of drugs on the mtFAO pathway and MRC activity.[10,151,153] However, for drugs inducing only macrovacuolar steatosis with no clinical report of microvesicular steatosis, investigations on mitochondrial function (and genome) should be completed with experiments exploring other metabolic pathways whose alterations can induce hepatic lipid accumulation. In particular, *in vivo* and *in vitro* investigations suggest that drug-induced inhibition of VLDL secretion could be a rather frequent mechanism leading to hepatocellular triglyceride accumulation.[69–71,73] This assumption seems to be also supported by ongoing experiments performed in our laboratory in human differentiated HepaRG cells showing that 7 out of 9 drugs inducing steatosis in this cell model at non-cytotoxic concentrations are able to inhibit the secretion of apolipoprotein B in the culture medium, thus suggesting impairment of VLDL synthesis and/or output. Among the 7 drugs, troglitazone was reported to be a potent inhibitor of hepatic triglyceride synthesis in rats,[166] while rifampicin has been suspected to impair VLDL formation and/or secretion in rodents.[175,176]

For drugs able to induce microvesicular steatosis, a severe inhibition of mtFAO is undoubtedly the main mechanism of small fat droplet accumulation. Because some drugs can induce macrovacuolar steatosis in some patients and microvesicular steatosis in others, it is surmised that the residual mtFAO flux might be substantial and low (or nil), respectively for macrovacuolar and microvesicular steatosis (Fig. 2). Although underlying congenital defects in enzymes involved in mtFAO and OXPHOS have been detected in a few patients,[3,10,13,75] further efforts should be done to look for other genetic predispositions (*e.g.*, SOD2 polymorphism) and non-genetic factors such as alcohol overconsumption and polytherapy. Because numerous drugs can impair mitochondrial function,[9,10,13–17,26,31,50] it is conceivable that polymedicated patients might be at risk for severe mitochondrial dysfunction and microvesicular steatosis. However, prospective clinical studies might be difficult to design and perform because of the low incidence of this liver lesion. It must also be underlined that drug-induced impairment of mtFAO is expected to curb a key compensatory metabolic pathway that could be set up during NAFLD in order to limit hepatic fat accumulation. Indeed, several experimental and clinical investigations reported higher hepatic mtFAO in the context of NAFLD.[23,177,178] Hence, NAFLD patients treated with drugs potentially impairing mtFAO might be at risk for fatty liver worsening.[73,171]

As already mentioned (see Section 4), it is still unknown why lipids (mainly triglycerides) accumulate preferentially as tiny and large droplets, respectively in drug-induced microvesicular and macrovacuolar steatosis. Nevertheless, in both types of hepatic steatosis, triglyceride accumulation could be an adaptive protection against the toxicity of some lipid derivatives such as palmitic and stearic acids, similar to what has been proposed in NAFLD.[179,180] It is noteworthy that although macrovacuolar steatosis is the main form of lipid accumulation in NAFLD, hepatocytes with microvesicular steatosis can also be present in some patients, in particular in those with more advanced histology of the disease.[181,182] However, in the context of NAFLD, it is still unclear whether hepatocytes with microvesicular steatosis present a profound mitochondrial dysfunction, similarly to what happens in Reye-like syndrome.[9,87] Although mitochondrial ultrastructural alterations were observed in NAFLD hepatocytes presenting microvesicular steatosis,[182] further studies will indeed be needed in order to determine whether the mtFAO pathway is severely impaired within these hepatocytes as in drug-induced Reye-like syndrome.

Conflict of interest

The author declares that he has no conflict of interest.

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