

Fatty liver and drugs: the two sides of the same coin

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Abstract. – Drug-induced liver injury (DILI) is a common and underestimated cause of liver disease. Several drugs and other xenobiotics can be the cause of different clinicopathologic patterns of liver disease. Steatosis and steatohepatitis are rare but well-documented types of DILI. Over the past decades commonly used drugs like amiodarone, tamoxifen, irinotecan, methotrexate, valproic acid and glucocorticoids have been recognized to be associated with steatosis. Even though the pathophysiological pathways are still only partially understood, inhibition of mitochondrial beta-oxidation, reduced very low-density lipoprotein secretion, insulin resistance induction and increased de novo synthesis or increased liver uptake of fatty acids are considered the main pathogenic mechanisms through which drugs can lead to hepatic steatosis. On the other hand, fatty liver itself is a very common clinical condition, and there is a growing awareness of the potential risk factors for DILI due to the underlying metabolic condition itself.

Key Words:

Drug induced liver injury, DILI, Fatty liver, NAFLD, Drugs.

Introduction

Drug-induced liver injury (DILI) is one of the more frequent causes of liver damage. The real incidence in clinical practice is unknown. Indeed, DILI often simulates any type of liver damage, and it's not always recognized¹. Liver damage due to drugs may vary and can act out every form of liver histology from steatosis to cirrhosis. Several retrospective studies have tried to assess the incidence of DILI by analyzing pharmacovigilance data or databases from pharmaceutical companies. Analysis of a Swedish outpatient hepatology

clinic database revealed that 6.6% of 1664 cases were consistent with DILI². In the United Kingdom, the incidence of DILI has been estimated at 2.4 cases per 100,000 persons³. Recently two prospective studies have been developed in Europe: in a French study the annual incidence of DILI was 13 cases per 100,000 persons⁴, while in an Icelandic study which excluded acetaminophen-associated liver injury, the annual incidence of DILI was 19 cases per 100,000 persons⁵.

In the United States DILI is the first cause of acute liver failure and causes 10% of all cases of acute hepatitis⁶. Interestingly, hepatotoxicity is the first cause of drug withdrawal from the global market^{6,7}; this evidence reflects the difficulty in gathering enough information about adverse liver effects before drug approval.

Several classifications of DILI have been proposed. The more useful in clinical practice identify three types of DILI: hepatocellular, cholestatic and mixed pattern (Table I)⁸.

Another classification may be based on histological findings. Although liver biopsy is not mandatory, it can be useful for better staging and grading of liver injury⁹. The DILI Network (DILIN) differentiated 18 histological damage patterns from analysis of liver biopsies from 249 patients with suspected DILI¹⁰. The five most common patterns of injury were acute and chronic hepatitis, acute and chronic cholestasis and cholestatic hepatitis; together they represented 83% of all cases. According to DILIN, steatosis (at least 5%) was highly prevalent in liver biopsies from patients with DILI: it was found in 65/249 patients (26.2%) who underwent liver biopsy for DILI. However, the data could be biased by a pre-existing fatty liver. Three rare but well-documented patterns are mainly characterized by the fatty liver: macrovesicular steatosis (no cases in DILIN database); mi-

Table I. Biochemical assessment of Drug-Induced Liver Injury.

Type of Injury	Biochemical Criteria
Hepatocellular	Isolated \uparrow SGPT ($\geq 2x$ ULN) or SGPT/ALP >5
Cholestatic	Isolated \uparrow ALP ($\geq 2x$ ULN) or SGPT/ALP <2
Mixed	\uparrow ALP and \uparrow SGPT and $2 < \text{SGPT/ALP} < 5$

SGPT, serum glutamate-pyruvate transaminase; ALP, alkaline phosphatase; ULN, upper limit of normal. (Modified by Watkins PB, Seeff LB. Drug-induced liver injury: summary of a single topic clinical research conference. *Hepatology*. 2006 Mar;43(3):618-31

crovesicular steatosis (1 case in DILIN database) and steatohepatitis (6 cases in DILIN database).

Fatty liver: a Subtype of DILI or Pre-existing Condition

Fatty liver is a common histological feature of several liver diseases. The rapid spread of obesity and diabetes is leading to a rapid increase of non-alcoholic fatty liver disease (NAFLD) prevalence and incidence^{11,12,13}. According to a recent meta-analysis of epidemiological data the global prevalence of NAFLD is estimated to be between 22% and 28% in the general adult population¹⁴; in Europe, the average prevalence rises from 26% in countries like Italy¹⁵ and Spain¹⁶ to 30.4% in Germany¹⁷. In his natural history, NAFLD can lead to severe hepatic complications (cirrhosis, hepatocellular carcinoma)¹² and extra-hepatic complications such as cardiovascular diseases¹⁸, chronic kidney disease¹⁹ and an increased risk of developing extra-hepatic tumours²⁰, particularly colorectal neoplasms²¹. The high prevalence of NAFLD and its natural history represent a serious threat for public health systems¹⁴, causing a burden on health²².

The high prevalence of NAFLD may explain the frequent finding of steatosis in liver biopsy of patients with DILI; however, drug-induced steatosis is a common event that should be kept in mind. Table II summarizes the drugs that have been associated with these histological subtypes of DILI.

According to the histological presentation, we can distinguish the following types of steatosis at liver histology:

Microvesicular Steatosis

Microvesicular steatosis is typically an acute liver injury histologically characterized by several small lipid vesicles inside the hepatocyte that leave the nucleus at the center of the cell. Clinically it can be associated with an increase of serum aminotransferase levels, lactic acidosis, and acute liver failure^{23,24}.

This type of cell damage is related to an acute mitochondrial dysfunction that leads to an impairment of beta-oxidation of fatty acids. Consequently, poorly oxidized fatty acids are stored as triglycerides in the hepatocyte cytoplasm giving shape to the typical histological pattern²⁵. Several drugs have been linked to microvesicular steatosis such as valproate acid, tetracycline, aspirin (Reye's syndrome), ibuprofen, nucleoside/nucleotide analogues (zidovudine) and vitamin A^{26,27}.

Macrovesicular Steatosis

Macrovesicular steatosis, in contrast to microvesicular, is histologically characterized by a unique large vacuole of fat that fills the whole hepatocyte, displacing the nucleus to the cell periphery. From a pathophysiological point of view, triglyceride accumulation in hepatocytes could develop for several reasons: 1) impaired beta-oxidation of fatty acids, 2) reduced very low-density lipoprotein (VLDL) secretion²⁸, 3) increased de novo synthesis or increased liver uptake of fatty acids²⁹, 4) insulin resistance induction³⁰.

Drugs that have been linked to macrovesicular steatosis include glucocorticoids, methotrexate, oestrogens, tamoxifen, chemotherapeutic agents (5-fluorouracil and cisplatin) and non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, and sulindac, as well as the anti-malarial drug mefloquine^{31,32,33}.

Drug-induced Steatohepatitis (DISH)

Steatohepatitis is histologically characterized by steatosis, lobular inflammation and hepatocellular injury (hepatocellular ballooning with or without Mallory hyaline bodies) and in some cases peri-sinusoidal fibrosis³⁴. The development of DISH can be considered as precipitation of pre-existing steatosis or as a de novo liver disease³⁵. The pathogenesis of DISH has not yet been entirely elucidated; nevertheless, oxidative stress seems to work as a key pathological mechanism³⁶. Mitochondrial dysfunction and inhibition of the mitochondrial respiratory chain (MRC) lead to increased production of reac-

tive oxygen species (ROS)^{37,38}. ROS elicit the peroxidation of fatty acids which leads to inflammation and fibrogenesis through activation of Kupffer and Ito cells³⁹.

ROS and reactive lipid peroxidation products can directly damage the MRC and mitochondrial DNA leading to a vicious cycle that results in a greater production of ROS. Furthermore, ROS can induce the nuclear translocation of NF- κ B and the production of several cytokines such as TNF- α , IL-8, and TNF- β which have chemotactic, pro-inflammatory and pro-fibrogenic roles⁴⁰. Finally, ROS, via NF- κ B nuclear translocation, cause hepatocyte expression of Fas ligand leading to Fas/Fas ligand-mediated apoptosis⁴¹. Drugs that have been associated with DISH encompass tamoxifen, amiodarone, perhexiline, propranolol, and valproic acid^{26,33,42}.

Drugs Responsible for Fatty Liver

The wide epidemiological impact of fatty liver disease and the diagnostic troubles regarding DILI make it even more difficult to identify a molecule as the origin of this subtype of the drug-induced liver disease. Even if several drugs may be responsible for DILI, few drugs have a proven causative role for steatosis.

Amiodarone

Amiodarone is a widely used anti-arrhythmic drug. It is a cationic amphiphilic compound capable of interfering with mitochondrial function, thus facilitating the onset of fatty liver⁴⁰. The unprotonated and lipophilic drug goes across the mitochondrial membrane and is then protonated and trapped inside the mitochondria. The high intra-mitochon-

drial drug concentration inhibits beta-oxidation and disrupts the electron transport chain leading, respectively, to fat accumulation and ROS production^{43,44}. Amiodarone can also inhibit microsomal triglyceride transfer protein (MTP) which physiologically plays a key role in the assembly of VLDL²⁸. Other drugs have a similar structure and could have the same effects on hepatocytes, i.e. perhexiline and diethylaminoethoxyhexestrol⁴⁵.

Mild and asymptomatic elevation of aminotransferase can be found in 4–80% of patients that take amiodarone, while a cholestatic biochemical pattern is rare⁴⁶. Even if rare events, severe acute hepatitis due to liver toxicity has been reported in a minority of cases (1–3% of patients) and a few cases of microvesicular steatosis have also been described³⁵. Furthermore, protracted use of amiodarone has been associated with progressive fibrosis even including cirrhosis^{47,48}.

In 2009 the FDA approved dronedarone, a ‘second generation’ of an anti-arrhythmic drug. In past years several case reports of liver injury associated with dronedarone have been published; two of them highlighted a severe acute hepatic failure that needed a liver transplant⁴⁹. Dronedarone is able to inhibit beta-oxidation of fatty acids *in vivo*⁵⁰, but it isn’t able to disrupt the electron transport chain *in vivo* probably because of its short half-life compared to amiodarone (13–19 hours and 15–142 days, respectively), which makes it more difficult to reach too high a concentration inside mitochondria⁴⁵.

Tamoxifen

Tamoxifen is a selective oestrogen receptor modulator widely used in the treatment of breast

Table II. Association between histological liver pattern of injury and drugs.

Drug	Macrovesicular steatosis	Microvesicular Steatosis	Steatohepatitis
Amiodarone (and CAD)	√		√
Chemotherapeutic agents (5-FU, irinotecan, oxaliplatin)	√		√ (irinotecan)
Tetracyclin		√	
Valproic Acid		√	
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)		√	
Methotrexate	√		√
Tamoxifen	√		√
ASA (Reye’s Syndrome)		√	
Steroids	√	√	
Cocaine		√	
Alcohol	√		
Total parenteral nutrition	√		

CADs, cationic amphiphilic compounds; 5-FU, 5-Fluorouracil; ASA, acetylsalicylic acid.

cancer, particularly in molecular subtypes that express oestrogen receptors (ER). Although their role is not completely understood, two subtypes of ER (ER-alpha and ER-beta) are both expressed in the mitochondrial membrane⁵¹. A recent study demonstrated that tamoxifen induces fatty liver disease through the impairment of beta-oxidation³⁸ and the promotion of de novo fatty acid synthesis⁵². From a molecular point of view tamoxifen is a cationic amphiphilic compound so, like amiodarone, drug accumulation is another important mechanism of liver toxicity⁴⁵.

Hepatic steatosis and, more rarely, steatohepatitis are the most common tamoxifen-induced liver diseases⁵³. Almost a third of patients develop steatosis, usually within 2 years from the beginning of anti-oestrogen therapy³³ and tamoxifen itself may accelerate the development and progression of NAFLD; in fact, obesity and other metabolic syndrome risk factors are themselves independent risk factors for tamoxifen-induced fatty liver disease⁵⁴. Even though steatosis and steatohepatitis rapidly improve after drug withdrawal⁵⁵, we must not forget the beneficial effect of tamoxifen for women with breast cancer. Improving metabolic control with medical therapy can ameliorate the fatty liver disease caused by this drug and could be the best compromise for women that cannot suspend oncologic therapy³⁵.

Raloxifene, another selective ER modulator, is used especially in post-menopausal women for the treatment and prevention of osteoporosis. It has been observed that it can worsen a pre-existing fatty liver disease; in vivo studies demonstrate that raloxifene is able to inhibit beta-oxidation of fatty acids^{38,56}.

Chemotherapeutic Agents

The term 'chemotherapy-associated steatohepatitis' (CASH) recently appeared in the literature; this reflects the increasing evidence of chemotherapeutic liver toxicity. Irinotecan, 5-fluorouracil (5-FU) and oxaliplatin are three of the most studied chemotherapeutic agents that have been associated with steatohepatitis. A lot of therapeutic regimens contain these three drugs, particularly those used for patients with metastatic colon cancer (FOLFOX regimen – 5-FU, leucovorin and oxaliplatin, FOLFIRI regimen – 5-FU, leucovorin and irinotecan – and FOLFIRINOX regimen – 5-FU, leucovorin, irinotecan, and oxaliplatin)⁴⁵.

A recent study tried to assess the chemotherapy-associated liver injuries in patients who underwent neoadjuvant chemotherapy for colorectal

liver metastasis, showing that steatosis is a common histological finding with different possible stages of damage²⁷. In a more recent study, the authors made a histological evaluation of non-tumoural liver parenchyma from 384 patients who underwent liver resection for metastatic colorectal cancer; 65% received preoperative chemotherapy for a median duration of 24 weeks. This study evidences that an increased BMI, administration of irinotecan and diabetes mellitus are associated with hepatic steatosis and steatohepatitis⁵⁷. Although the mechanisms are still unknown, irinotecan seems to have the most steatogenic potential compared to the other two drugs⁵⁸. Considering that irinotecan inhibits topoisomerase 1, preventing the recoiling of DNA, some authors hypothesized that it could also affect mitochondrial DNA leading to mitochondrial toxicity, which is known to play a pivotal role in drug-induced fatty liver⁴⁵.

Methotrexate (MTX)

MTX is a commonly used drug with chemotherapeutic and immunosuppressant properties. Since the 1980s it has been known that MTX-associated liver toxicity is strictly related to the large cumulative dose that is usually reached both in rheumatologic chronic low dose regimens and in oncologic high dose cyclic regimens. In fact, Kremer et al⁵⁹ showed that a polyglutamated metabolite of MTX is progressively stored inside hepatocytes, causing liver toxicity. Furthermore, MTX is able to determine mitochondrial dysfunction by depleting mitochondrial folate stores; in particular, it cannot directly affect intra-mitochondrial folate storage but hampers folate entry into the mitochondria, limiting the replenishment of mitochondrial folate stocks⁶⁰. It has been demonstrated that MTX-related mitochondrial dysfunction leads to ROS generation and induction of caspase-dependent apoptosis⁶¹⁻⁶⁴.

Recently, several studies focused on another interesting pathophysiological mechanism of liver damage: MTX can disrupt the intestinal epithelial barrier⁴⁵ leading to leaky gut syndrome which is known to be associated with the onset and progression of the fatty liver disease⁶⁵.

The MTX-associated liver injury is clinically characterized by mild to moderate aminotransferase elevations in up to 50% of patients⁶⁶. This biochemical alteration is usually transient; sometimes it can require dose regulation or drug discontinuation⁶⁷. Steatohepatitis, significant fibrosis and cirrhosis are the most concerning pathological patterns that may be caused by MTX; it has

been estimated that only 4-5% of patients develop these advanced diseases⁶⁸. Besides the capability to induce steatohepatitis, MTX can also worsen the pre-existing fatty liver disease, leading to a progressive liver disease. Indeed, several risk factors have been associated with MTX-induced hepatotoxicity including NAFLD, alcohol consumption, chronic hepatitis B or C (HBV, HCV), obesity and diabetes⁵⁵. The American Academy of Dermatology's guidelines for the management of psoriasis developed a strict recommendation for MTX hepatotoxicity in psoriasis⁶⁹.

Valproate

Valproate, or valproic acid, is a commonly prescribed anti-epileptic and anti-psychotic drug. It is a branched-chain fatty acid so it competes with other fatty acids in hepatocyte metabolic pathways⁴⁵. The free acid form of valproate enters the cell and then the mitochondria where it is conjugated with coenzyme A (CoA). The resulting lack of CoA hampers the beta-oxidation of fatty acids leading to triglyceride storage and to steatosis⁷⁰. The mitochondrial toxicity of valproate also arises from its ability to release protons and consequently disrupt electron chain transport and ATP generation⁷¹. Furthermore, chronic valproate therapy induces systemic insulin resistance and weight gain, increasing the risk of progression of a pre-existing fatty liver disease^{55,72}.

Several clinical studies have been developed about valproate liver toxicity. In a recent study hepatic steatosis, assessed by ultrasound scan, was found in 61% of patients exposed to valproate²⁶. Mild elevation of aminotransferase without cholestasis is common among treated patients⁷³. From a histological point of view, valproate usually causes progressive microvesicular steatosis; this further supports the mitochondrial impairment due to this drug³⁴. Liver injury associated with valproic acid usually recovers with drug suspension or dose reduction. Although valproate-related severe idiosyncratic hepatotoxicity is rare, there are cases of acute liver failure or even death reported in the literature, especially among the paediatric population⁷⁴⁻⁷⁶.

Tetracycline

Although several antibiotics are hepatotoxic, tetracycline is the main class known to be a potential cause of fatty liver disease, especially if administered intravenously, while adverse reactions after oral administration are quite rare⁷⁷⁻⁸⁰.

Microvesicular steatosis is the characteristic histological pattern of liver injury that can result

from tetracycline-based therapy³⁴. This pathological feature reflects the molecular mechanisms of damage that these drugs may trigger. In fact, tetracycline inhibits beta-oxidation of fatty acids and MTP, an enzyme that plays a key role in the assembly of VLDL²⁸. A recent study pointed out that tetracycline can also decrease the expression of peroxisome proliferator-activated receptor alpha (PPAR α), carnitine palmitoyltransferase I (CPT-I) and fatty acid binding protein 1 (FABP-1) which are genes involved in beta-oxidation⁸¹. Furthermore, doxycycline and minocycline, which are tetracycline compounds, have been shown to be able to enhance ROS production in hepatocytes⁸². This evidence seems to depend on the activation of activating transcription factor 4 (ATF4) which up-regulates CYP2E1 leading to the generation of ROS⁴⁵. These molecular mechanisms of hepatocyte injury seem to be confirmed in an interesting study that investigated the proteomic profile in a tetracycline-treated murine model. The authors highlighted increased oxidation of some mitochondrial proteins involved in beta-oxidation of fatty acids, such as long-chain specific acyl-CoA dehydrogenase, leading to a slowdown of their enzymatic activity⁸³.

Nucleoside Reverse Transcriptase inhibitors (NRTI)

In last few years, NRTIs have substantially changed the natural history of HIV infection. Anti-retroviral therapy (ART) is based on the association of two drugs with different mechanisms of action. Although all classes of anti-retroviral agents (NRTI, protease inhibitors – PI – and non-nucleoside reverse transcriptase inhibitors – NNRTIs) have shown potential hepatotoxicity, NRTIs are most commonly associated with liver damage resembling steatosis⁸⁴, especially in obese patients and women⁸⁵. The NRTI class of drugs also includes didanosine, stavudine, lamivudine, emtricitabine, abacavir and tenofovir. Zidovudine (AZT), the first ART drug to be approved and commercialized, has been widely studied for its adverse liver effect. AZT and other NRTIs, except lamivudine, may cause both microvesicular and macrovesicular steatosis⁸⁵. Cases of acute liver failure, microvesicular steatosis and lactic acidosis have rarely been reported⁸⁷.

Besides inhibition of viral reverse transcriptase, these drugs have been shown to be able to inhibit human DNA polymerase γ , the enzyme responsible for the replication of mitochondrial DNA. This effect leads to mitochondrial toxic-

ty and subsequent oxidative stress and accumulation of fat^{88,89}. Another mechanism of damage recently proposed is the inhibition of autophagy which leads to accumulation of dysfunctional mitochondria, increased ROS production, increased apoptosis, decreased proliferation and increased intracellular lipid accumulation^{90,91}.

Conclusions

Several drugs are known to be associated with DILI, and their number will probably increase due to the continuous approval and market placement of new molecules, and also to the improved awareness of both physicians and healthcare systems about this issue. It has been established that drugs commonly used in clinical practice (i.e. MTX, tamoxifen and chemotherapeutic agents) may cause any kind of NAFLD/NASH-like histological modification. The other side of the same coin is that the underlying metabolic condition causing a fatty liver may exacerbate the risk of DILI. Thus, considering the high prevalence of NAFLD and the high use of drugs with potential hepatotoxic risk, monitoring of liver function and identification of clinical signs suggestive for liver damage should be kept in mind.

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