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EDITORIAL

Biochemical mechanisms in drug-induced liver injury: Certainties and doubts

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Abstract

Drug-induced liver injury is a significant and still unresolved clinical problem. Limitations to knowledge about the mechanisms of toxicity render incomplete the detection of hepatotoxic potential during preclinical development. Several xenobiotics are lipophilic substances and their transformation into hydrophilic compounds by the cytochrome P-450 system results in production of toxic metabolites. Aging, preexisting liver disease, enzyme induction or inhibition, genetic variances, local O_2 supply and, above all, the intrinsic molecular properties of the drug may affect this process. Necrotic death follows antioxidant consumption and oxidation of intracellular proteins, which determine increased permeability of mitochondrial membranes, loss of potential, decreased ATP synthesis, inhibition of Ca^{2+}-dependent ATPase, reduced capability to sequester Ca^{2+} within mitochondria, and membrane bleb formation. Conversely, activation of nucleases and energetic participation of mitochondria are the main intracellular mechanisms that lead to apoptosis. Non-parenchymal hepatic cells are inducers of hepatocellular injury and targets for damage. Activation of the immune system promotes idiosyncratic reactions that result in hepatic necrosis or cholestasis, in which different HLA genotypes might play a major role. This review focuses on current knowledge of the mechanisms of drug-induced liver injury and recent advances on newly discovered mechanisms of liver damage. Future perspectives including new frontiers for research are discussed.

Key words: Adverse effects; Apoptosis; Drug toxicity; Liver diseases; Microsomes; Mitochondria; Necrosis

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INTRODUCTION

Drug-induced liver injury is the leading cause of acute liver failure and transplantation in western countries. The detection of subtle mechanisms that lead to potential drug hepatotoxicity is of key importance and remains a major challenge in clinical practice.

The frequent involvement of the liver in drug-induced toxicity depends on its anatomical location (the liver is the primary port of entry for ingested drugs) and its physiological and biochemical functions because of the abundance of metabolizing enzymes.

The spectrum of injury secondary to drug reaction ranges from mild damage to massive hepatic destruction. However, if one considers the large consumption of drugs, the latter possibility is rather infrequent[1]. While direct toxic damage is dose-dependent, predictable and experimentally reproducible, idiosyncratic damage is rather supported by the innate and the adaptive immune system. With few exceptions of intrinsic hepatotoxicity, most cases of drug-induced liver injury are idiosyncratic. Toxicity can be experimentally tested by administering the compound at increasing doses, in the presence of

metabolic inducers or inhibitors or toxicity enhancers, with depletion of protective systems, or by co-administering the drug with a known toxic compound. In general, *in vitro* tests precede *in vivo* experiments. Intracellular organelles and their functions are often the primary targets of hepatotoxicity[2]. Not only hepatocytes, but also cholangiocytes, Kupffer cells, Ito cells and sinusoidal endothelial cells can be involved in the process of drug-induced hepatotoxicity. Some drugs can induce cholestasis by impairing bile secretion or by causing obstruction of extrahepatic bile ducts[3].

This review deals with the main mechanisms associated with drug-induced hepatic injury, by discussing current views on intra- and extracellular mechanisms of damage and cell death with respect to different drugs. Future perspectives on emerging problems, namely liver steatosis and genetic polymorphisms, are also discussed.

RISK FACTORS

As toxicity is exerted mostly through metabolites rather than the parent drugs, factors affecting metabolite formation are of key importance. Accordingly, genetic polymorphisms and environmental influences on metabolizing enzymes play an important role. Of note, drug-induced hepatotoxicity occurs mainly in women[4], and this points to the existence of hormonal conditioning factors. Additional genetic, metabolic and immunological factors also may have a role in idiosyncratic hepatotoxicity. All such mechanisms can occur if specific metabolic pathways are activated and previous exposure has sensitized the organ with the formation of specific antibodies (e.g. halothane). In addition, the intrinsic toxicity of some molecules can depend on the expression of genetic variants, as occurs for paracetamol[5]. Although preexisting liver disease generally is believed to play a minor role as a risk factor for hepatotoxicity, there are some well-documented exceptions. Hepatotoxicity caused by isoniazid, for example, is more common among patients with viral hepatitis and/or human immunodeficiency virus (HIV) infection[6]. Patients undergoing antiretroviral treatment for HIV infection are at higher risk for severe hepatotoxicity when co-infected with hepatitis B or C viruses, particularly if therapy includes protease inhibitors[7]. Fatty liver is another condition that is particularly prone to stress damage[8]. Further studies are needed urgently in this respect, linking toxic injury to liver steatosis, which is becoming an emerging health problem, because of the increasing epidemic of obesity and diabetes as part of the metabolic syndrome[9].

GENERAL MECHANISMS OF DAMAGE

Although major pathways leading to drug-induced liver injury include necrosis and/or apoptosis, a net distinction between these two processes is sometimes difficult and both events often coexist in the same microscopic field[10]. Several factors may influence the hepatocyte response to a toxic insult and the extent of damage results from the intervention of intrinsic and extrinsic cell factors. A combination of age, sex, genetics, hormones, cell energetic status, underlying liver disease, environmental factors, and local O_2 supply, strongly contributes to the expression of cell death mediators[11]. Less frequently, hepatocyte injury follows on from vascular damage as a consequence of the occlusion of the centrilobular vein (i.e. azathioprine, estrogens, progesterone, pyrrolidine alkaloids). Generally, hepatocytes react to toxic aggression by activating defense mechanisms that include hypertrophy of the endoplasmic reticulum, induction of protective systems (glutathione, GSH), and synthesis of heat shock and acute phase proteins.

Apoptosis and necrosis initially may follow common metabolic pathways. When the injury affects the maintenance of functional cell programs, hepatocytes preferentially die *via* apoptosis, thus limiting the extent of the injury. Necrotic damage generally begins at the cytoplasmic level and thus involves mitochondria and the nucleus in determining swelling and loss of plasma membrane integrity. It becomes irreversible when cytosolic Ca^{2+} concentration increases[12,13] for increased release by mitochondria and endoplasmic reticulum, or increased extracellular influx. Apoptosis determines cytoplasmic and nuclear condensation and fragmentation without loss of membrane integrity. Drug-induced apoptosis is generally spotty, whereas necrosis is zonal.

The mechanisms of damage include interference with hepatic transport proteins (i.e. organic anion transporting polypeptides), bile salt export pump, or with the nuclear receptor-mediated regulation of drug metabolism and transport[14,15].

MECHANISMS OF CELL DEATH

Hepatocyte death typically follows an apoptotic or necrotic pathway[16], mainly depending on predisposing factors[10]. General mechanisms of hepatotoxicity include reactive metabolite formation, antioxidant depletion, and protein alkylation. Intracellularly generated signaling can activate B-cell CLL/lymphoma 2 (Bcl-2) family members (Bax and Bid) which form pores in the outer mitochondrial membrane. This condition favors the release of intramembrane proteins and promotes chromatin condensation and DNA fragmentation. Alternatively, mitochondrial dysfunction, through reactive oxygen species (ROS) delivery and peroxynitrite formation, triggers membrane permeability transition and leads to membrane potential collapse with decrease of energy production and release of nucleases[17].

Apoptosis

Apoptosis results from an ATP-dependent death program that is characterized by activation of specific pathways involving death ligands and death receptors (e.g. Fas ligand with Fas) with activation of the caspase cascade (Figure 1). There are two different activating pathways of drug-induced hepatocyte apoptosis. The "intrinsic way" is triggered by intracellular signals

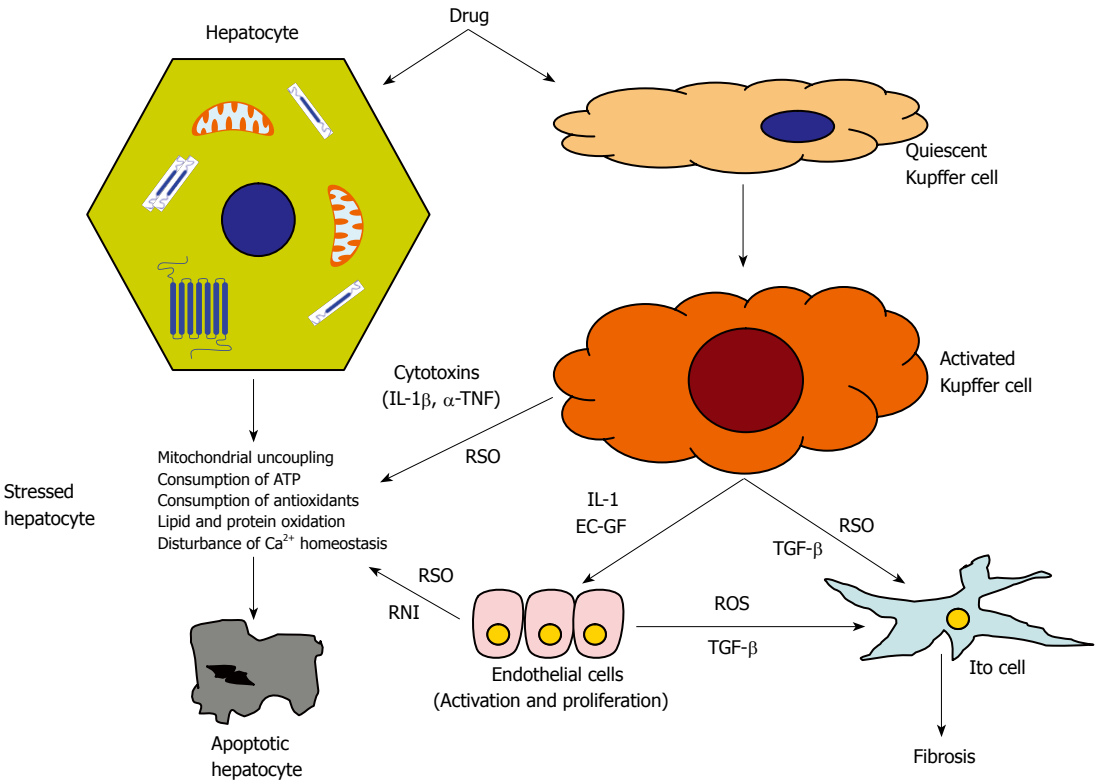

Figure 1 Schematic representation of subtoxic damage of hepatocyte in response to moderate dose of drug. Drug molecule activates Kupffer cells is metabolically processed by hepatocytes. These events may result in hepatocyte stress which is worsened by the intervention of reactive oxygen species (ROS) and nitrogen species from activated endothelial cells. Final result is apoptotic death and Ito cells activation with promotion of fibrosis. EC-GF: Endothelial cell growth factor; IL1: Interleukin 1; IL1β: Interleukin 1β; RNI: Reactive nitrogen intermediates; ROS: Reactive oxygen species; TGF-β: Transforming growth factor β; TNF: Tumor necrosis factor α.

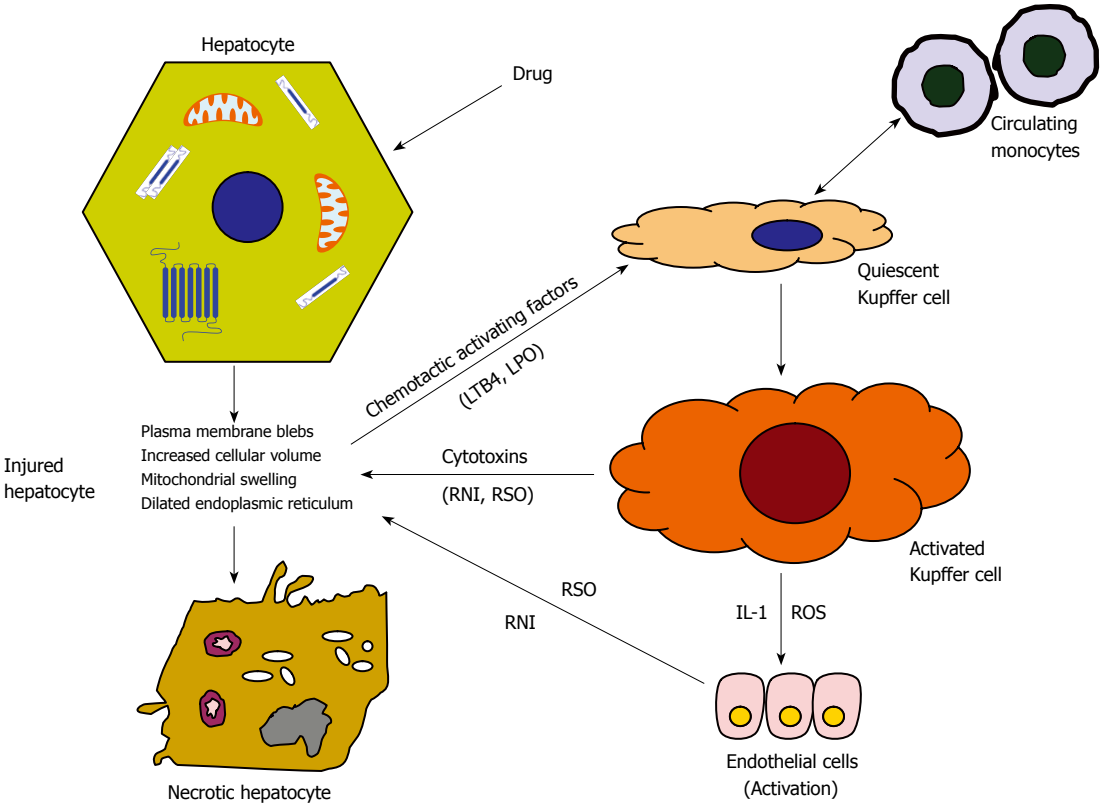

Figure 2 Schematic representation of toxic damage of hepatocyte in response to high dose of drug. High drug amount is processed by hepatocytes with production of reactive metabolites which induce cell injury. Toxic products and chemotactic factors released by damaged hepatocytes stimulate the activation of Kupffer and endothelial cells with a subsequent delivery of reactive oxygen (ROS) and nitrogen species. The intracellular damages result in necrotic death. LPO: Lipid peroxidation; LTB4: Leukotriene B4.

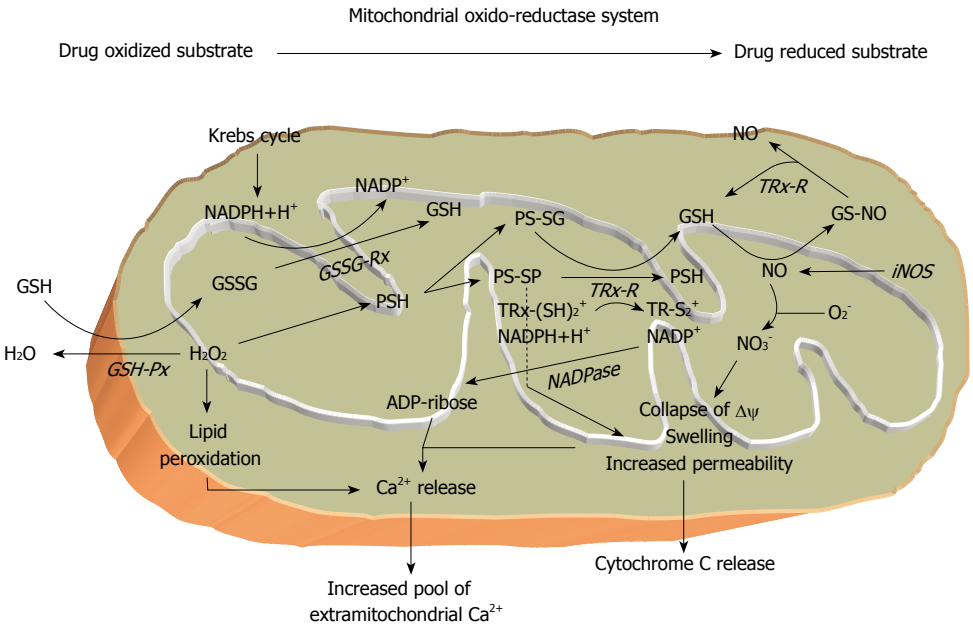

Figure 3 Schematic representation of mitochondrial oxido-reductase system. Several drug molecules directly or after metabolic release of toxic intermediates can cause mitochondrial alterations at different levels. The following impairment of the energetic and redox balance finally triggers apoptotic or necrotic processes according to a poor or sufficient ATP level. Important regulatory mechanisms rely on the glutathione dependent redox status of proteins. GSH: Reduced glutathione; GSSG: Oxidized glutathione; GSH-Px: Glutathione peroxidase; GSSG-RX: Glutathione reductase; iNOS: Inducible nitric oxide synthetase; NO: Nitric oxide; PSH: Protein sulphydrils; PS-SG: Protein mixed disulfides; PS-SP: Protein-protein disulfides; TRx: Thioredoxin; TRx-R: Thioredoxin reductase; TR-S_2: Oxidized thioredoxin.

scattered directly by the drug or its metabolites, with activation of a cascade of reactions that damage nuclear and/or mitochondrial DNA directly. Single-stranded DNA subsequently will stimulate intracellular sensor systems and induce the expression of the effector p53. In the "extrinsic way", new surface antigens on hepatocyte membranes work as receptors. The interaction with ligands, such as tumor necrosis factor alpha (TNF-α) or Fas, activates cytotoxic T cells and liver non-parenchymal cells, with release of cytokines[18] that engage death receptors on the cell surface[19]. After binding, the receptor trimerizes and leads to a clustering of death domains. Intrinsic and extrinsic ways finally promote the activation of interleukin (IL)-1β converting enzyme, which activates caspases and nucleases.

Generally, hepatocytes are resistant to TNF-α-induced cytotoxicity[20]. In fact, under normal conditions, the activation of membrane receptors stimulates the synthesis of anti-apoptotic molecules and enzymes (e.g. Bcl-2, NO synthase), mediated by the intervention of the nuclear transduction factor nuclear factor-κB (NF-κB). Therefore, increased cell sensitivity to TNF-α or to other specific ligands is required to trigger subsequent events[21]: a strong signaling response with activation of the executioner caspases[22], and the involvement of mitochondria to amplify death mechanisms in the presence of a poor caspase activation[23].

Necrosis

Drug-induced cell necrosis results from an intense and massive perturbation of cell homeostasis, with ATP depletion (Figure 2) associated with cytoskeletal alterations, cellular swelling and bleb formation[16]. The next steps include lysosomal breakdown, bleb rupture, and irreversible collapse of electrical and ion gradients.

When a high amount of toxicant reaches the liver, necrosis occurs because of dramatic intracellular alterations, or as a consequence of oxygen and nitrogen radical attack from activated Kupffer and endothelial cells[24]. On this occasion, drugs are oxidized by the cytochrome P-450 (CYP-450) enzymes, with release of a large amount of reactive metabolites, with promotion of lipid and protein oxidation and depletion of GSH. Oxidized proteins and protein adducts may have immunogenic properties and activate Kupffer and polymorphonuclear cells, with subsequent release of ROS. The formation of protein disulfides results in increased permeability of the inner mitochondrial membrane, with loss of membrane potential, decrease of ATP synthesis, inhibition of Ca^{2+}-dependent ATPase, decreased capability to sequester Ca^{2+}, oxidation of actin, microfilament breakage, and membrane bleb formation[25].

Abnormal control of cell volume is a major factor that promotes hepatocyte necrosis. Oxidative stress, very fast consumption of cellular energy, and mitochondrial dysfunction activate anaerobic glycolysis, which results in decreased intracellular pH. The incoming acidosis is contrasted partially by H^+/Na^+ and Na^+/HCO_3^- exchanges with influx of Na^+. As a result of low ATP availability, Na^+ cannot be further exchanged and accumulates within the cell. The consequent osmotic load results in cell swelling and blocks the apoptotic process, which requires a reduction of the cell volume. This osmotic stress is worsened by the increase of cytosolic Ca^{2+} and results in plasma membrane rupture[26].

Additional mechanisms include nucleotide alterations

and protein synthesis disruption. In most cases, these actions follow drug-induced mitochondrial injury. However, discriminatory nucleotide alterations and oxidation of protein sulfhydryls (γ-glutamyl synthetase and glucose 6-phosphate dehydrogenase) are promoted selectively by some drugs; one example is the damage to the ATPase complex observed after cisplatin intoxication[27].

CELLULAR AND INTRACELLULAR TARGETS OF DRUG HEPATOCELLULAR INJURY

Aspects to discuss include non-parenchymal hepatic cells, microsomes, mitochondria, and nuclear receptors. Much evidence suggests the participation of non-parenchymal hepatic cells in drug-induced hepatocellular injury[28], which depends on factors such as the intrinsic characteristics of the drug, its dose, its metabolites, and the local O_2 supply[29]. Activation of Kupffer cells results in the release of inflammatory mediators and ROS, and modulates hepatocyte injury[30]. It has been shown that inhibition of macrophage activation or administration of TNF-α antagonists protects hepatocytes against paracetamol toxicity[31], and that depletion of Kupffer cells attenuates thioacetamide hepatotoxicity[32]. Indeed, both Kupffer and endothelial cells can be activated secondarily by chemotactic factors (i.e. leukotriene B4) released by injured hepatocytes[24,33], which in turn, can be damaged by TNF-α and IL-1β released from activated non-parenchymal cells. Examples of drug hepatotoxicity that involves non-parenchymal cells are that seen with methotrexate (activation of hepatic stellate cells to myofibroblasts, and liver fibrosis may develop even in the absence of liver enzyme elevation); bosentan (inhibition of transport proteins including the bile salt export pump[34]); sulindac (competitive inhibition of canalicular bile salt transport, a contributing factor to cholestatic liver injury[35]); cyclophosphamide and azathioprine (sinusoidal obstruction syndrome, veno-occlusive disease, follows a severe depletion of GSH in sinusoidal endothelial cells. This damage results in fibrosis of the hepatic sinusoids).

Microsomes are another target of hepatocellular damage induced by drugs. Biotransformation of lipophilic drugs *via* CYP-450 metabolic pass and the subsequent excretion of their metabolites are essential to avoid intracellular accumulation of toxic compounds. Less than 10 CYP-450 enzymes accounts for > 90% of all drug oxidation. Most adverse drug reactions depend on the release of reactive metabolites and ROS, which may overwhelm lethal insult, sensitize the innate immune system, or haptenize, thus eliciting immunoallergic reactions[36]. If metabolites have a particularly high reactivity, they can even bind and inactivate the metabolic enzymes[37]. This occurs with drugs that show a narrow therapeutic index (e.g. terfenadine and astemizole). Several factors may affect the efficiency of the microsomal metabolism: namely aging, liver disease, enzyme induction and inhibition, genetics (existence of slow and fast acetylators), and O_2 supply. Changes in the level of CYPs may have a dramatic impact on drug metabolism. P-450 enzymes are subjected to multiple levels of regulation and expression; the latter being dominant in zone 3 just surrounding the centrilobular vein. Expression of P-450 isoforms varies with age; therefore, the capacity for drug metabolism is a function of age[38,39]. Polymorphisms in P-450s or induction/inhibition account for the appearance of adverse reactions. In this regard, it has been noted that the constitutive androstane receptor (CAR) binds drugs and regulates the expression of the genes that code for CYP3A and CYP2B[40]. Also, induction or inhibition of CYPs by herbal remedies accounts for the increasing number of case reports of hepatotoxicity[41]. In fact, some herbal components are converted to toxic metabolites by P-450 enzymes; this is the case of aristolochis acid, which generates the highly reactive cyclic nitrenium ions[42]. Upregulation of specific P-450 enzymes has been described during rifampicin treatment[43] in experimental models of obesity and fatty liver[44] and in humans with nonalcoholic fatty liver disease (NAFLD)[45].

Mitochondria are often a major target of drug toxicity, and therefore mitochondrial dysfunction represents a major determinant of hepatotoxicity[46,47] (Figure 3). Indeed, mitochondria are the gateway at which signals that initiate cell death converge[3,48]. By integrating signaling networks, mitochondria have an active role in several metabolic pathways[49]. Signals may damage mitochondria directly or act indirectly by activating death receptors. In particular, reactive metabolite formation, GSH depletion and protein alkylation are associated with mitochondrial dysfunction, and represent critical initiating events for drug-induced toxicity. Opening of pores in the outer mitochondrial membrane, release of proteins and cytochrome c, imbalance in intracellular Ca^{2+} homeostasis, and intracellular accumulation of Na^+ are essential steps in hepatocyte death[17,50]. In this context, the maintenance of the mitochondrial GSH pool[21,51] is important to detoxify ROS and maintain the reduced status of membrane protein sulfhydryls, including the ATP synthase complex and the Ca^{2+}-dependent ATPase. A fall of total cellular GSH below 15% (< 1 μmol/g) inevitably is associated with lethal cell damage by involving the mitochondrial stores[52,53]. Common events that lead to apoptosis and necrosis act through mitochondrial permeabilization and dysfunction. In particular, it seems that the number of mitochondria that undergo pore opening is associated with apoptosis or necrosis, according to ATP availability or deficiency[47]. Some drugs exert toxic effects on mitochondria only after their metabolic activation at the microsomal level (isoniazid/rifampicin), after inducing endoplasmic reticulum stress (paracetamol) or even lysosomal dysfunction. The study of these mechanisms has revealed intriguing relationships between mitochondria and other intracellular organelles[54-56].

Recent advances in molecular biology have revealed

that nuclear receptors such as the pregnane X receptor (PXR) and CAR act as intracellular sensors for lipophilic compounds by encoding proteins and regulating the expression of enzymes[57,58] that are involved in drug oxidative metabolism, disposition and transport[15]. Their incorrect activation may result in drug metabolism disturbance. PXR can be activated or inhibited by a variety of structurally different drugs. Its activation is associated with downregulation of several genes[59] that influence mitochondrial ketogenesis[60] and favor mitochondrial imbalance and hepatic steatosis[61]. These receptors also represent important drug targets. In fatty livers, peroxisome proliferator-activated receptor (PPAR) activation/deactivation is particularly important, not only for the switch from simple steatosis to steatohepatitis, but also for maintaining the efficiency of specific metabolic drug pathways[62]. PPARs and other oxidative stressors can be activated also by macrophage-released molecules (i.e. Stat-3 and NF-κB)[63]. The existence of single-nucleotide polymorphisms is associated in humans with drug transport alterations as a predisposing factor for drug-induced cholestasis[14].

COMMON PATHWAYS OF DRUG-INDUCED LIVER DAMAGE

Immune system

The liver is a site of intense immunological activity and represents a tolerogenic immune organ for lymphocytes. Activation of Kupffer cells, and recruitment of macrophages and immune cells result in inflammation and injury caused by cytokines release[64]. These events are major factors in initiating and maintaining drug-induced liver injury[65].

The drug itself and its metabolites can activate an immune response in the liver: the molecule is processed by antigen-presenting cells in the central lymphoid tissue directly, or after the appearance of haptens or new antigens on the hepatocyte membrane. The latter case follows a covalent binding of the drug molecule with membrane constituents or intracellular proteins[66]. This hypothesis is supported by the observation that neutrophil depletion protects against paracetamol toxicity[67]. Also, idiosyncratic reactions are more likely to occur in the presence of an inflammatory state[68]. Effectors are dendritic cells, which act by sensing pathogens and triggering adaptive immune responses. These responses are characterized by activation of B lymphocytes, which release immunoglobulins and kinines and activate the complement cascade, and of T lymphocytes, which produce lymphokines (CD4) or determine direct cytotoxicity (CD8) *via* surface-molecule expression and the release of mediators (e.g. perforin and granzyme)[69]. As a consequence, inhibition of lymphocyte activation reduces the extent of drug-induced hepatocyte injury[70].

The local O_2 supply has an important role in the progression of immune-mediated toxic liver injury. For example, metabolism of halothane under the anaerobic conditions of the reductive pathway may result in mild hepatitis, whereas, in the presence of a high O_2 supply, the oxidative pathway may induce massive liver necrosis[29]. These different effects may be explained by the higher immunogenicity of oxidized metabolites that form adducts with proteins. This example suggests the potential capacity of some drugs to trigger autoimmune hepatitis in some patients. In fact, statins, hydralazine and procainamide may trigger autoimmune reactions in predisposed patients[71]. Most of these patients are positive for HLA-DR3, 4 or 7, which are known to be associated with increased risk of autoimmunity. Halothane toxicity rarely occurs after first exposure; but antibodies against CYP 2E1-mediated trifluoroacetylated metabolite-protein adducts can be detected after frequent exposures to halothane.

Direct toxicity

Paracetamol hepatotoxicity is the classical example of direct liver injury. Given at recommended doses, paracetamol is generally safe, but its intrinsic toxicity at higher doses represents the most important cause of acute liver failure and transplantation. Predominantly metabolized by conjugation with sulfate and glucuronide (metabolites are excreted into bile by Mrp2 and extruded into blood through Mrp3), only a small amount is degraded by CYP 2E1 to the highly reactive metabolite *N*-acetyl-benzoquinoneimide (NAPQI). NAPQI is, in turn, detoxified by binding with GSH. If the amount of paracetamol that reaches the liver exceeds 12-15 g, the conjugating capacity is overwhelmed and the remaining unbound NAPQI covalently binds to cellular and mitochondrial proteins, which leads to cell necrotic death. In the presence of CYP 2E1 hypertrophy and/or decreased GSH availability (e.g. chronic alcoholism, malnutrition, and prolonged intake of barbiturates), NAPQI formation is increased even at therapeutic doses, and after overwhelming the GSH stores, it may cause severe liver injury[72,73].

Events start with disturbances of intracellular Ca^{2+} homeostasis, with an increase in cytosolic Ca^{2+} levels, Bax and Bid translocation into mitochondria, and mitochondrial oxidative changes with accumulation of oxidized GSH and peroxynitrite[74,75]. The latter induces membrane permeability transition, with collapse of mitochondrial membrane potential, inability to synthesize ATP, release of mitochondrial proteins with calpain activation, and release of cytochrome C and endonucleases. ATP deficiency prevents caspase activation but induces nuclear DNA damage, and activates intracellular proteases that lead to cell membrane rupture and hepatocyte necrosis[76,77]. These intracellular events explain the massive cell death and liver failure observed after paracetamol poisoning[17]. The recent observation that paracetamol toxicity is modulated by CAR gives rise to new concepts that are important for the general understanding of drug-induced liver injury[78]. Accordingly, the presence of gene polymorphisms may explain inter-individual differences

in susceptibility to paracetamol toxicity. Finally, a role for hepatic non-parenchymal cells in paracetamol-induced hepatocellular injury also has been suggested. In fact, the chemical elimination of Kupffer cells by gadolinium chloride has been observed to reduce the extent of paracetamol-induced liver injury[31].

Direct toxicity of the liver is also induced by another drug, valproate, a branched medium-chain fatty acid with eight carbon atoms. Its chronic intake is associated with weight gain and it causes insulin resistance and NAFLD in 61% of treated patients[79]. Mechanisms of toxicity rely on mitochondrial β oxidation inhibition followed by the appearance of microvesicular steatosis[80]. Mitochondrial dysfunction follows the microsomal production of toxic metabolites (4-ene-valproate, 2,4-diene-valproate)[81], decreased activity of complex Ⅳ of the respiratory chain, and depletion of coenzyme A (CoA) and carnitine[80]. Preexisting mitochondrial impairment or deficiency of cofactors involved with valproate metabolism (e.g. carnitine) may represent risk factors for hepatotoxicity[82].

Idiosyncratic reactions

Unpredictable idiosyncratic reactions can follow the administration of virtually any drug. As a consequence, an enormous number of hepatic reactions have been registered for practically all drug classes. Several mechanisms have been elucidated, including TNF-α-induced apoptosis, inhibition of mitochondrial function, and neoantigen formation. Here, we report some of the most representative cases. Hepatotoxicity associated with the non-steroid anti-inflammatory drug (NSAID) nimesulide has led recently to its commercial withdrawal in some countries[83]. The mechanism is unknown, although liver histology has shown centrilobular and bridging necrosis[84]. Diclofenac potentially leads to zone 3 necrosis, autoimmune hepatitis, or even cholestasis[85] in predisposed individuals. The major pathway of diclofenac metabolism is through 40-hydroxylation by CYP 2C9[86]. Diclofenac also undergoes oxidative metabolism by CYP 2C8 to form reactive diclofenac acyl glucuronide and 5-hydroxydiclofenac[87]. Nucleophilic displacement can then replace the glucuronic acid moiety to form adducts with free cysteine thiols[88], and act as a potential hapten that triggers autoimmunity. Studies with diclofenac-protein conjugates have shown that diclofenac-treated hepatocytes carry antigens that are recognized by T-cell- and non-T-cell-enriched splenocytes[89]. As a consequence, changes in the activity of CYP 2C8, its haplotype distribution, or impairment in the clearance of acyl glucuronide may potentially increase the risk of hepatotoxicity. Polymorphisms, such as the presence of UGT2B7*2 allele, favor the development of diclofenac hepatotoxicity[90].

EXAMPLES OF LIVER DAMAGE INDUCED BY COMMONLY USED DRUGS

Aspirin induces hepatotoxicity that is different from that of other NSAIDs. Aspirin is hydrolyzed into salicylic acid, which is transformed actively by mitochondria into its salicyl-coenzyme A derivative. This compound indirectly inhibits the β oxidation of long-chain fatty acids and increases NADH availability, thus resulting in increased capacity of mitochondria to decarboxylate branched chain amino acids[91,92]. The negative effect on mitochondrial β oxidation probably is augmented by concomitant viral infection that affects mitochondrial function. This combination may determine microvesicular steatosis known as Reye's syndrome[93].

Nefazodone, a triazolopyridine trazodone, an antidepressant drug, recently has been withdrawn from the market because of hepatotoxicity. Mechanisms include inhibition of mitochondrial respiratory complex Ⅰ and Ⅳ, associated with accelerated glycolysis. This effect leads to mitochondrial membrane potential collapse, GSH depletion and oxidative stress[94].

Hepatotoxicity exerted by isoniazid, an anti-tuberculosis drug is related to its metabolite monoacetyl hydrazine, which is activated at the CYP-450 level and detoxified by N-acetyltransferase 2. These enzymes undergo genetic variability and environmental alterations; slow acetylator status and CYP 2E1 genetic polymorphism are risk factors for isoniazid hepatotoxicity[95,96]. Concomitant therapy with rifampicin, a CYP-450 inducer, significantly increases the risk of liver injury[56].

Amiodarone is a commonly used antiarrhythmic drug that consists of a benzofuran ring coupled with two iodine and diethyl-ethanolamine side chains substituted with a p-OH-benzene structure. Amiodarone accumulates within mitochondria and causes toxicity by inhibiting state 3 glutamate and palmitoyl-CoA oxidation and by decreasing mitochondrial respiration[55]. Electron transport chain complexes and β oxidation are also inhibited by amiodarone[96]. The chemical structure of benzarone resembles that of amiodarone. Benzarone, a non-halogenated benzbromarone derivative, is used for the treatment of vascular disorders. It decreases mitochondrial membrane potential, as well as state 3 oxidation and respiratory control ratio, uncouples oxidative phosphorylation, and inhibits β oxidation. Benzarone increases the production of ROS, as well as the leakage of cytochrome C, with final induction of mitochondrial permeability transition[97].

Troglitazone, a PPAR agonist, causes hepatocyte injury by dissipating the mitochondrial transmembrane potential, which favors superoxide generation, thioredoxin oxidation and activation of the kinase-1-dependent apoptosis signaling pathway[98].

HIV-1 protease inhibitors are essential components of antiretroviral therapy. However, mitochondrial toxicity represents a serious problem for patients taking antiretroviral drugs. It occurs most often with administration of a full dose of ritonavir and saquinavir[7]. Genetic HLA variants of the immune system seem to participate in the hepatotoxicity induced

Table 1 Mechanisms that favor high sensitivity of fatty liver to drug toxicity and necrotic cell death

Initial change	Intermediate effects	Consequences
Increased bioactivation (microsomal CYP 450s)	Higher amount of toxic metabolites	Consumption of antioxidants
	Increased release of ROS	Lipid peroxidation
Mitochondrial dysfunction	Decreased energy production (ATP) and cytochrome c content	Over-expression of uncoupling protein 2
		Increased Ca^{2+} efflux
	Increased release of ROS and NO derivatives	Protein oxidation and nitration
	Pores opening and increased membrane permeability	Expression of FAS ligands
		Calpain activation and protein cleavage
Impaired intracellular signaling and trafficking	Alterations of nuclear receptors and sensors	Defective transcription of repair mechanisms
	Increased DNA fragmentation rate	
Activation of non-parenchymal cells (Kupffer cells) and enzymes	Increased release of transforming growth factor-β1, p53, TNF-α	Inflammation and pro-oxidant attack
	Increased NADPH oxidase activity	

ROS: Reactive oxygen species; TNF: Tumor necrosis factor.

by abacavir, another antiretroviral drug. Co-infection with hepatitis viruses is known to increase the risk of mitochondrial toxicity induced by these nucleoside compounds[6].

CHOLESTASIS

Hepatic clearance of drugs depends on the activity of transport proteins that are located on the hepatocyte canalicular membrane. Alterations of these transporters by drugs or genetic polymorphisms increase the susceptibility to cholestatic injury[14]. As a consequence, cholestasis is one of the most important features of drug-induced hepatotoxicity[99]. Substrates for hepatic transport proteins include indomethacin, statins, digoxin, enalapril, midazolam, tamoxifen, diclofenac, methotrexate, and troglitazone. Selective inhibition of ATP-dependent bile salt transport proteins represents an additional mechanism of damage; therefore, co-administration of drugs at this level may enhance the risk of cholestasis. Examples are troglitazone plus lisinopril, itraconazole and verapamil, bosentan and glyburide[100,101]. Changes in the expression of drug transporters in conditions of chronic liver disease can also result in marked alterations in drug disposition[102]. Examples are increased bioavailability of drugs with high hepatic extraction, and decreased hepatic clearance of drugs with a low hepatic extraction and of those with biliary excretion[103]. Finally, cholangiocytes can also be damaged directly by drugs. Flucloxacillin, an isoxazolyl-penicillin, can cause cholestasis by injuring bile duct epithelial cells[104].

FUTURE PERSPECTIVES

Drug-induced liver injury occurs when the organ defense systems are overwhelmed. Preexisting conditions may contribute to the extent of damage. Two examples in this respect are the existence of fatty liver disease (liver steatosis), and genetic polymorphisms.

The mechanisms that favor high sensitivity of fatty liver to drug toxicity and necrotic cell death are depicted in Table 1. It is known that fatty liver has a reduced tolerance towards stress conditions, i.e. ischemia-reperfusion, prolonged fasting, and exposure to *t*-butyl-hydroperoxide[105,106]. Potential mechanisms that favor increased susceptibility of steatotic liver to drug-induced toxicity include mitochondrial imbalance[107], increased mitochondrial ROS production[108], and deficient repair capacity[109]. Indeed, a high incidence of hepatotoxicity has been observed in patients with type 2 diabetes[110], a condition that is associated inevitably with fatty liver[111]. Therefore, it is conceivable that hepatotoxic drugs might produce injury even at non-toxic doses in patients with fatty liver, although in a recent study[112], steatosis appeared to protect against paracetamol toxicity through preserving microcirculatory alterations. Defective hepatobiliary transport as well as the downregulation of Mrp2, as observed in rats with fatty liver, may represent additional predisposing factors for damage in these organs[113].

Genetic polymorphisms are another important issue. Polymorphisms of CYP-450s account, at least in part, for the variability of efficacy and for the occurrence of adverse drug reactions, and may explain the variety of effects exerted by the same drug in different subjects. Genetic variations in the glutathione S-transferases (GSTT1 and GSTM1) have been associated with drug-induced hepatotoxicity[114]. Subjects who display mutations in some alleles that code for manganese superoxide dismutase have a higher risk of developing drug-induced liver injury[115]. Genetic mitochondrial abnormalities are a major determinant of the high susceptibility towards idiosyncratic liver injury caused by drugs that target mitochondria, especially in aged and female subjects[116]. Genetic polymorphisms associated with alteration of hepatobiliary transporters have implications in drug-induced cholestasis[14].

CONCLUSION

The search for the underlying mechanisms of damage is expected to lead to new intriguing perspectives for diagnosing and treating toxic liver injury. Today, certain microsomal and mitochondrial metabolic pathways can be assessed easily *in vivo* by performing breath tests with

substrates that release CO_2 during their metabolism. Methionine and α-ketoisocaproate breath tests assess mitochondrial functions and are altered after exposure to alcohol or drugs, thus reflecting specific metabolic alterations induced by exogenous compounds[92,117]. Such noninvasive diagnostic tools may guide evaluation of the effect of therapeutic strategies.

Future issues might include the use of cytokine and death receptor antagonists, strategies directed at factors that cause mitochondrial damage, and approaches that promote survival gene expression that may overcome drug-induced cell death. In this regard, toxicogenomics, a combination of toxicology and genomics, attempts to identify the effects of drugs on gene expression, and the role of genetic polymorphisms in drug-induced liver injury. However, although recent developments in genetics and toxicology have provided some new insights into drug hepatotoxicity, the complex interactions of hepatotoxins with genetic and environmental risk factors responsible for the onset of toxic injury have yet to be elucidated. Severe drug-induced liver diseases therefore remain unpredictable for most drugs. The identification of new risk factors and a better understanding of pathogenetic mechanisms will certainly have implications for health care and pharmaceutical developments in the near future.

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