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Mechanisms of Drug Induced Liver Injury

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Synopsis

Drug induced liver injury (DILI) represents a broad spectrum of liver manifestations. However, the most common manifestation is hepatocyte death following drug intake. DILI can be predictable and dose dependent with notable example of acetaminophen toxicity. Idiosyncratic DILI occurs in an unpredictable fashion at low frequencies implying that environmental and genetic factors alter the susceptibility of individuals to the insult (drugs). An biochemical stress is usually initiated by drugs and their reactive metabolites through covalent binding or direct damage to mitochondria, which leads to oxidative stress, activation of stress signaling pathways, impairment of mitochondrial function, endoplasmic reticulum stress, etc. The ultimate cell death pathways converges at mitochondria through acting on mitochondrial outer-membrane permeability (MOMP) or mitochondrial permeability transition (MPT). The striking HLA associations with idiosyncratic DILI highlight the critical role of the adaptive immune response in pathogenesis, which is now believed to be unmasked in genetically susceptible individuals by the biochemical stress in the liver triggered by drug and/or metabolites. The drug-induced biochemical stress may also contribute to the severity of injury by sensitizing hepatocytes to the lethal effects of the immune response. Adaptive mechanisms including antioxidant signaling (such as Nrf2 signaling) , mitophagy, autophagy, unfolded protein response, anti-inflammatory and immune tolerance dampen and ameliorate injury. All together, the development and severity of injury is determined on the battle between the hazardous stress and adaptive responses within the hepatocytes and the innate and adaptive immune systems.

Keywords

Idiosyncratic drug-induced liver injury; Cell death; Reactive metabolites; Oxidative stress; Stress signaling; Mitochondria; Adaptive immunity; HLA associations

Drug-induced liver injury (DILI) occurs in an incidence of 10 to 15 in 10, 000∼100,000 in USA[1, 2], yet it has caused remarkable fatality from acute liver failure yearly. Acetaminophen (APAP) alone accounts for the half of the overall cases of acute liver failure in USA[3, 4]. DILI can also mimic all forms of acute or chronic liver diseases (hepatitis, cholestasis or a mixed)[5], which is often under-recognized due to the complexity of clinical scenarios. Most of DILI cases are idiosyncratic. A threshold dose (50 to 100mg) may be required for DILI to occur[6, 7]. When the dose threshold is exceeded, injury occurs in very small number of individuals. Although acetaminophen hepatotoxicity is dose dependent, idiosyncratic injury

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has been observed with a lower dose (less than 4 gram per day) depending on individual susceptibility (i.e. alcohol intake, fasting).

Over the past decades, much effort has been put into research to understand the mechanisms of hepatotoxicity and explore biomarkers for DILI surveillance. The central question of pathogenesis is how a drug or its metabolites initiate and propagate cell death within the liver with the assistance of surrounding immune cells.

Necrosis, Apoptosis and Necroptosis

The fundamental process in DILI is the death of hepatocytes (in some circumstances, cholangiocytes or endothelial cells) in the background or recruitment of inflammation. DILI manifests clinically with hepatocellular injury, cholestasis or a mixture of both.

Necrosis and apoptosis, theoretically, are two distinct modes of cell death. The fundamental differences between necrosis and apoptosis are the integrity of the plasma membrane, and involvement of caspase activation. Apoptosis is a sterile, “clean” programmed cell death characterized by cell shrinkage and chromatin fragmentation. The rapid removal of apoptotic cells by phagocytes or other cells minimizes the surrounding inflammation. Mechanistically, apoptosis is mediated by ATP-dependent intracellular proteolytic cascades involving caspases. This is triggered by the extrinsic pathway related to death ligand/ receptors binding at plasma membrane, or intrinsic pathways triggered by oxidative stress, radiation, DNA damage or toxins leading to increasing permeability of mitochondrial outer membrane. Idiosyncratic DILI is mainly mediated by the innate or adaptive immune response involving death ligands such as TNF-α and FasL. TNF-α and FasL bind to death receptors of hepatocytes, triggering their demise of apoptosis.

Necrosis involves cell swelling, membrane bleb formation and eventually the rupture of plasma membrane. The release of cellular components from necrotic cells elicits an inflammatory response. Necrosis is considered an oncotic lysis caused by loss of ion homeostasis as a result of severe mitochondria dysfunction and profound ATP depletion.

Necrosis was conventionally considered as an incidental, “unwanted” cell death in a non-regulated manner. Increasing evidence has shown that necrosis can be tightly regulated. One of the remarkable observations is that when an apoptotic pathway was initiated upon TNF-α/ TNFR binding in L929 cells[8], inhibition of apoptosis with caspase inhibitors or ATP depletion leads to the shifting of cell demise to necrosis. Many terms have been used to categorize this type of cell death, such as necroptosis, programmed necrosis, regulated necrosis, etc. It involves activation of receptor-interacting protein kinases[9, 10]1 and 3 (RIP1 and RIP3), and participation of mitochondria. This cell death has been implicated in pathophysiology of many diseases such as acute pancreatitis[11], brain injury[12], and viral infection[13, 14].

Acetaminophen-induced cell death of hepatocytes has characteristic morphologic changes of necrosis. Mechanistically, the reactive metabolite, N-acetyl-p-benzo-quinoneimine (NAPQI), leads to profound mitochondrial GSH depletion, covalent binding, severe impairment of mitochondrial function and cessation of ATP production, which lead to disruption of ion homeostasis and consequently oncotic necrosis. Interestingly, many stress kinases such as c-Jun N-terminal kinase (JNK)[15], glycogen synthase kinase-3b (GSK-3β)[16], apoptosis signal-regulating kinase-1 (ASK1)[17], mixed-lineage kinase-3 (MLK3)[18], PKC[19], RIP1(unpublished from our lab) have been found to actively regulate the process. This suggests that APAP-induced necrosis might be a programmed necrosis.

The Involvement of Reactive Metabolites

The fact that liver is the central organ for drug metabolism places it as a prime target for reactive metabolites of drugs. In most cases, drugs or their reactive metabolites are detoxified via phase II conjugation (glucuronidation, acetylation, sulphation, glutathione conjugation, etc) and excreted out of cells through multi-drug resistance-associated protein (MRP) transporters (phase III). Reactive metabolites are often produced through oxidation and reduction by cytochrome P450 (Phase I). A balance between production and detoxification/transport are critical in determining the most upstream aspects of DILI, namely exposure of hepatocytes to some threshold level of reactive chemicals. Dose and lipophilicity (favoring hepatic distribution) are key additional factors in determining achievement of a threshold exposure to initiate hepatocellular stress or injury. DILI is often primed by reactive metabolites and their covalent binding to cellular proteins. Inhibition of bile salt export pump (BSEP) may aggravate hepatotoxicity[20, 21] not only by causing cholestasis in some cases, but more importantly by bile acid retention causing mitochondrial and endoplasmic reticulum (ER) stress which may amplify injury or sensitize hepatocytes to other injury mechanisms.

APAP metabolism has been well characterized. APAP is predominantly metabolized through sulphation and glucuronidation. A small proportion of APAP is oxidized by cytochrome P450 isoform 2E1 (to lesser extent 1A2 and 3A4) to a reactive form, N-acetyl-p-benzo-quinoneimine (NAPQI). NAPQI readily attacks free thiols, leading to rapid and selective glutathione (GSH) depletion in cytosol and mitochondria. When GSH is depleted, NAPQI covalently binds to thiol groups of cellular and mitochondrial proteins causing mitochondria dysfunction, and the production of mitochondrial reactive oxygen species (mROS), MAP kinase activation and downstream events leading to necrosis (see below).

Oxidative stress and covalent binding activate not only toxic signaling, but also protective and adaptive pathways. One such pathway is nuclear factor erythroid 2-related factor 2 (Nrf2)/Keap1 signaling[22, 23]. Nrf2 is usually maintained at a very low level in the cytosol as newly synthesized Nrf2 is rapidly bound to Kelch-like ECH-associated protein 1 (Keap1), an adaptor of Culin E3 ligase, which shuttles Nrf2 to Culin E3 ligase complex for ubiquitin proteasomal degradation[24]. Keap 1 has 25 cysteine residues and acts as a redox and electrophilic sensor. The Keap-1 homodimer binds to a single Nrf2 molecule at DLG motif with a low affinity and ETGE motif with a high affinity[25, 26]. According to “hinge and latch” model[25, 26], when thiols of Keap1 are oxidized or covalently bound to electrophiles such as NAPQI, the conformation of Keap1 changes and Nrf2 dissociates from DLG binding site while remaining bound to Keap1 at ETGE motif. Keap1 is thus occupied by Nrf2 that is not further ubiquitinated. This allows *de novo* newly synthesized Nrf2 to translocate to the nucleus where it binds to the antioxidant response element (ARE) promoters and activate the transcription of many antioxidant genes including glutamate-cysteine ligase (GCL), thioredoxin reductase, peroxiredoxin and glutathione S-transferase[27, 28]. This increases glutathione synthesis and ROS detoxification. Meanwhile, increased expression of MRPs by Nrf2 activation enhances the export of drugs/metabolites out of cells.

Involvement of Stress Signaling

C-Jun N-terminal kinase (JNK) activation in response to TNF-α is usually rapidly dampened by NF-κB transcription of survival genes. Thus the activation is transient and often nontoxic. Sustained JNK activation, however, leads to lethal consequences. This has been extensively studied in APAP mouse model. Inhibition of JNKs with a small synthetic molecule (SP600125) or silencing of JNK expression with siRNA protects against APAP

hepatotoxicity[15, 29]. JNK is a family of serine/threonine kinases belonging to the MAPK family. Upstream is MAP3K (e.g. ASK1 or MLK3) that phosphorylates and activates MAP2K (e.g. MKK4/7) which in turn phosphorylates JNK. Knockout of ASK1 blunts JNK activation and attenuates APAP toxicity[17]. Silencing GSK-3β or MLK3 blunts early phase of JNK activation and also exhibits a similar protective effect[16, 18].

JNK can be activated by many stressors such as ROS, UV light and cytokines. In APAP models, mitochondrial ROS seems to play a crucial role in prolonged JNK activation. We observed that sustained JNK activation occurred upon profound GSH depletion and covalent binding in mitochondria in APAP model. This might at least be achieved by modifying several redox-sensitive regulators of JNKs such as thioredoxin (Trx), GSH S-transferase Pi (GST-Pi) and JNK phosphatases. ASK1 is held inactive by Trx at physiologic conditions and Trx oxidation allows the dissociation of ASK1 for activation[30]. GSH S-transferase Pi (GST-Pi) interacts directly with JNK as an inhibitor in non-stressed cells. ROS induces polymerization of GST-Pi via intermolecular disulfides, causing dissociation of GSTr polymer from JNK[31].

A crucial downstream target of JNK is mitochondria. Sustained, activated JNK was found to translocate to mitochondria, further impairing mitochondrial function, and amplifying oxidative stress. This self-amplifying process eventually leads to collapse of mitochondrial function and cell death. Our lab has identified an important mitochondrial outer membrane protein Sab (SH3 domain-binding protein that preferentially associates with Btk) which binds JNK and mediates its effect on mitochondria[32]. Silencing Sab expression abolished sustained JNK activation, blocked JNK translocation and attenuated APAP toxicity. The details of how Sab, once phosphorylated by JNK, mediates inhibition of the electron transport (↑TROS), is currently not known.

The Involvement of Mitochondrial Dysfunction

The mechanisms of hepatocyte apoptosis and necrosis converge on mitochondria. The release of cytochrome C, apoptosis-inducing factor (AIF) and Smac from the mitochondrial intermembrane space are crucial to activate caspases and execute apoptosis in the presence of adequate ATP[33]. This requires permeabilization of the outer mitochondrial membrane (OMM). Necrosis occurs via mitochondrial permeability transition (MPT). MPT is composed of voltage-dependent anion channel (VDAC) from OMM, adenine nucleotide translocase (ANT) from the inner mitochondrial membrane (IMM), and cyclophilin D (CypD) from the matrix. This putative pore spans the mitochondrial outer and inner membranes. Its opening dissipates the proton gradient, resulting in the collapse of mitochondrial membrane potential and cessation of ATP production. As consequence, mitochondria swell and OMM ruptures releasing pro-apoptotic factors[34]. However, necrosis is the more likely the outcome in the setting of MPT opening, since MPT causes profound ATP depletion and MPT usually occurs in the context of oxidative stress which inactivates caspases.

Selectively permeabilizing OMM allows the release of the pro-apoptotic factors from the intermembrane space without disrupting IMM. Mitochondrial outer membrane permeability (MOMP) is primarily governed by Bax and Bak, which oligomerize and insert into the outer mitochondrial membrane to create pores for the release of cytochrome c and Smac/Diablo. Bax/Bak is regulated by pro-survival Bcl-2 members (Bcl-2, Bcl-XL, and Mcl1) and pro-apoptotic BH3-only members (Bim, Bid, Puma, Bad and Noxa)[35, 36]. The pro-survival factors such as Bcl-2 and Bcl-XL, directly inhibit Bax/Bak, whereas, pro-apoptotic factors such as tBid and Bim directly activate Bak/Bax, or de-repress Bak/Bax through binding and inhibiting Bcl-2 and Bcl-XL.

Programmed necrosis is considered as an “aborted” apoptosis. When an innate immune response is activated by lipopolysaccharide (LPS), TNF-α is released and binds to the membrane receptors, promoting cell death cascades, particularly JNK signaling. Necrosis occurs when the cell death signaling propagates upon ligand binding (TNF-α/TNFR) at the cytoplasmic membrane, but fails to execute apoptosis while the executioner caspases are suppressed with inhibitors such as Z-VAD-FMK. The caspases destroy RIP1 and RIP3. Recent studies[37, 38] showed that when caspases are inhibited under these conditions, RIP1/RIP3 form a complex which translocates to mitochondria where it activates mitochondrial fission necessary for cell death.

ROS/RNS generated by mitochondria are crucial in the mitochondrial death pathway in APAP model. This has been clearly demonstrated with a regioisomer of APAP, 3′-hydroxyacetanilide (AMAP)[39]. AMAP has a comparative metabolic profile to APAP, but the attack of its reactive metabolite spares mitochondria. With AMAP treatment, mitochondrial GSH is preserved and no hepatotoxicity occurs[40, 41]. The unsaturated lipid at mitochondrial membrane, such as cardiolipin, is particularly vulnerable to mROS attack. Cardiolipin collaborates with Bax polymer to promote OMM opening[42]. Some researchers[43] also showed that cardiolipin retains cytochrome c at IMM through electrostatic interaction. Cardiolipin peroxidation abrogates this association and frees cytochrome c, a necessary step for the release of cytochrome c to execute apoptosis. Release of mROS is able to activate JNK signaling in the cytoplasm as noted above. JNK translocates to mitochondria and leads to a self-amplifying cycle of JNK activation. Sustained JNK activation can alter the balance of Bcl-2 family by activating pro-apoptotic members and inactivating anti-apoptotic members or lead to sufficient ROS generation to cause MPT opening (Fig.1).

Unfit mitochondria may influence the susceptibility to drug-induced liver injury. This is exemplified by heterozygous mouse knockout of superoxide dismutase 2 (Sod2+/-) which exhibits greater vulnerability to liver injury from drugs such as troglitzone[44] and flutamide[45] as well as APAP[46]. SOD2(MnSOD) resides solely in mitochondrial matrix and regulates mitochondrial redox by scavenging superoxide. Null knockout (Sod2-/-) is not viable. The heterozygous knockout has preserved GSH/GSSG level, glutathione peroxidase and catalase at birth, but exhibits cumulative mitochondrial oxidative stress over time. With extrinsic insults (such as a drug) to mitochondria, the threshold of mitochondrial damage and/or mROS is lowered to elicit apoptosis or necrosis.

The Involvement of Mitochondrial Adaptation

Mitochondria provide primary energy source for the cell to function and cope with stress. Cyclical fusion and fission coupled with mitophagy are key in maintaining quality control and mitochondrial fitness[47]. Mitochondrial fission is mediated by Drp1, a large GTPase in the dynamin family. Drp1 is recruited from cytosol by a group of adaptor proteins including Mff, Mid49 and Mid51, and constricts both OMM and IMM at the site where mitochondria make contact with endoplasmic reticulum. Mitochondrial fusion involves OMM fusion and IMM fusion. OMM fusion is mediated by Mfn1 and Mfn2. IMM fusion is mediated by Opa1.

Many environmental insults or drugs (tetracycline, amiodarone, valproate and various antiviral nucleoside analogues) can directly damage mitochondria and deplete mitochondrial DNA. Inhibition of the electron transport chain leads to accumulation of reducing equivalents which generate ROS. Damage from ROS, such as oxidation of mitochondrial proteins, lipids and DNA, builds up within mitochondria. DILI related to mitochondrial toxicity is generally characterized by microvesicular steatosis, focal necrosis and cholestasis. The number of mitochondria in these cases is decreased. This represents a special case when

the drug or metabolite directly targets mitochondria and selective mitochondrial dysfunction is the cause of the phenotype. This should be distinguished from examples where the immune system is playing a major role in inducing the injury and the effects of the drug/metabolite on mitochondria influence the susceptibility or severity of immune (innate or adaptive)-mediated killing.

Mitochondrial fusion-fission is a self-repair mechanism by which the damage to mtDNA/proteins/lipids is dissipated by fusion with healthy mitochondria and cumulative damage is contained for elimination. Fusion allows component exchange/sharing of healthy mitochondria with damaged ones, thus rescuing stress and mitigating damage. The cumulative damage would eventually pose harm to the cells. Elimination of the damaged ones is necessary to further maintain quality control. Two major enzymes: PINK1 and PARKIN coordinate and flag the damaged ones for autophagic degradation[48]. The hypothesis is that the damaged components aggregate at the tip of mitochondria. Upon mitochondrial depolarization, PINK1, a membrane kinase, concentrates on the outer membrane of dysfunctional mitochondria. PINK1 recruits PARKIN, an E3 ligase, which ubiquintinates outer-membrane proteins for proteosomal and autophagic degradation[49-51]. Mitochondrial fission then allows the segregation of damaged parts which are subsequently engulfed by the autophagosome for elimination.

When the damage is overwhelming and/or repair system is severely impaired, stressed cells commit suicide. There is a close link between mitochondrial fission and cell death(both apoptosis and necrosis). Bax is found to co-localize with Drp1 and Mffs[52]. Oligermerization of Bax (to promote MOMP) is accompanied by Drp1-dependent fission. Lack of Drp1 delays cell death by decreasing cytochrome C release[53]. However, inhibition of Drp1 (sequestered in cytoplasm) also protects against necrosis, e.g. APAP and necroptosis models. The link between JNK, ROS, MOMP, MPT with Drp1 is not completely understood. There have even been suggestions that partial membrane remodeling without full fission is implicated in cell death.

The Role of Adaptive Immunity and Innate Immunity in Dili

Some DILI cases (For example: sulindac, phenytoin, and amoxicillin-clavulanic acid) have classic features of an allergic reaction such as a rash, fever and eosinophilia. The hypothesis is the drug or its metabolites act as haptens and covalently bind to a liver protein such as cytochrome p450. The drug-protein adducts are further processed in the macrophage/dendritic cell and presented as an antigen in complex with major histocompatibility complex (MHC) class II molecules, triggering the adaptive immune response by binding to T cell receptors of CD4 cells. This leads to CD8 cytotoxic T-cell activation. The sensitized CD8 T cells express FasL, TNF-alpha, and perforin that mediate cell death of hepatocytes. Although, most idiosyncratic DILI cases lack features of the systemic allergic reaction, adaptive immunity is believed to play a pivotal role in initiation and propagation of liver injury[54]. Several genome-wide association studies have revealed striking HLA haplotype associations with DILI. HLA-DRB1*1501 allele has a strong association with Augmentin-induced cholestatic liver injury[55-57]. The same allele is also associated with an increased risk of lumiracoxib-related hepatitis[58]. In the case of flucloxacillin, there is a strong link between HLA-B*5701 and DILI[59]. The carriers of this specific allele have a 80-fold increased risk in developing liver injury. Striking HLA haplotype associations suggest that DILI occurs as a consequence of a genetic predisposition targeted at adaptive immune response and antigen presentation/recognition. However haptenization alone is not sufficient to trigger the injury. Intrahepatic or extrahepatic stress caused by inflammation, infection or oxidative stress is believed to co-stimulate the adaptive immune response as well as to predispose hepatocytes for immune-mediated cell death.

Following some extent of initial cell death, the released cellular content of dead cells may activate innate immune cells including Kupffer cells, infiltrating monocytes and neutrophils in a paracrine fashion. High mobility group box 1 (HMGB1), as well as heat shock proteins and DNAs, are released from necrotic hepatocytes [60, 61]. These molecules have been termed damage-associated molecular patterns (DAMPS). DAMPs are able to bind to toll-like receptors of innate immune cells and promote the production of the cytokines such as TNF-alpha, IFNγ and IL-1 which could further modulate the intracellular events. Hepatic inflammation is frequently observed in DILI. It is conceivable these pro-inflammatory cytokines sensitize hepatocytes to biochemical stress, or regulate the adaptive immune-mediated cell injury. Trovafloxacin causes idiosyncratic liver injury in human, but is nontoxic to mice. Co-administration of LPS and trovafloxacin renders mice susceptible to severe hepatic necrosis[62, 63]. This suggests that an innate immune response could mediate DILI. However, clinical relevance of this model is unclear since the injury caused by this drug in humans appears to involve the adaptive immune system. The role of innate immune response in APAP-induced hepatic necrosis is quite controversial. It is believed that innate immunity is more likely beneficial in the clearance of necrotic cells and promoting tissue repair.

Some drugs, especially biologic immune modulators can activate underlying autoimmune hepatitis. In addition, a few other drugs (e.g. minocycline, nitrofurantoin) appear to cause a rare form of IDILI indistinguishable from autoimmune hepatitis, but responsive to drug withdrawal [64]. These cases represent an interesting liver manifestation as a result of interaction between drugs and the host immune system.

Conclusions

Idiosyncratic DILI is the result of the interplay between the environment, drugs and host (genetic, age, sex, immune factors, pre-existing diseases etc.) (Fig.2). Idiosyncratic DILI is often mediated by the adaptive immune response. Meanwhile, some drugs and metabolites can directly damage mitochondria, produce ROS and alter signaling pathway. To defend against the hazards induced by drugs, hepatocytes exhibit adaptive mechanisms including upregulation of Nrf2 signaling, mitophagy and autophagy to cope with stress. Furthermore, the innate and adaptive immune systems can adapt to dampen the response. Ultimately, the battle between hazardous and adaptive responses determines the development of severe injury, restoration of the liver after mild injury (so-called adaptation) or no injury at all.

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Idiosyncratic DILI is the result of the interplay between the environment, drugs and host (genetic, age, sex, immune factors, pre-existing diseases etc.)

Idiosyncratic DILI is often mediated by the adaptive immune response. Meanwhile, some drugs and metabolites can directly damage mitochondria, produce ROS and alter signaling pathway.

To defend against the hazards induced by drugs, hepatocytes exhibit adaptive mechanisms including upregulation of Nrf2 signaling, mitophagy and autophagy to cope with stress.

The innate and adaptive immune systems can adapt to dampen the response.

The battle between hazardous and adaptive responses determines the development of severe injury, restoration of the liver after mild injury (so-called adaptation) or no injury at all.

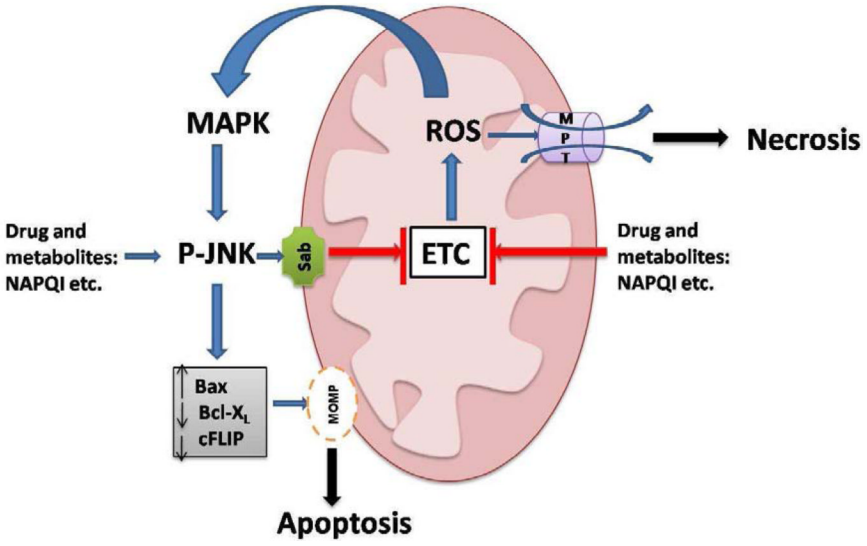

Fig. 1.

c-Jun N-terminal kinase (JNK) signaling and mitochondrial involvement in the death of hepatocytes. Sustained JNK activation and mitochondrial reactive oxygen species (ROS) generation are critical to induce cell death, particularly in the APAP model. In the APAP model, profound glutathione (GSH) depletion and covalent binding of NAPQI, lead to generation of mitochondrial ROS which activates mitogen activated protein kinases (MAPK) including apoptosis signal-regulating kinase-1 (ASK1) and mixed-lineage kinase-3 (MLK3). Both ASK1 and MLK3 activate MKK4/7 which in turn phosphorylates and activates JNK. Phosphorylated JNK translocates to mitochondria where it binds to and phosphorylates Sab, a mitochondrial outer membrane scaffold protein. This somehow leads to further impairment of electron transport chain (ETC) and enhancement of mitochondrial ROS generation. This self-amplifying process leads to sustained JNK activation and overwhelming production of mitochondrial ROS in the APAP model. As a result, mitochondrial permeability transition (MPT) collapses ATP production and necrosis occurs. In the model of tumor necrosis factor-a (TNF-a)-TNF/D-galactosamine, sustained JNK activation leads to mitochondrial outer membrane permeabilization (MOMP) and apoptosis by modulating the Bcl2 family.

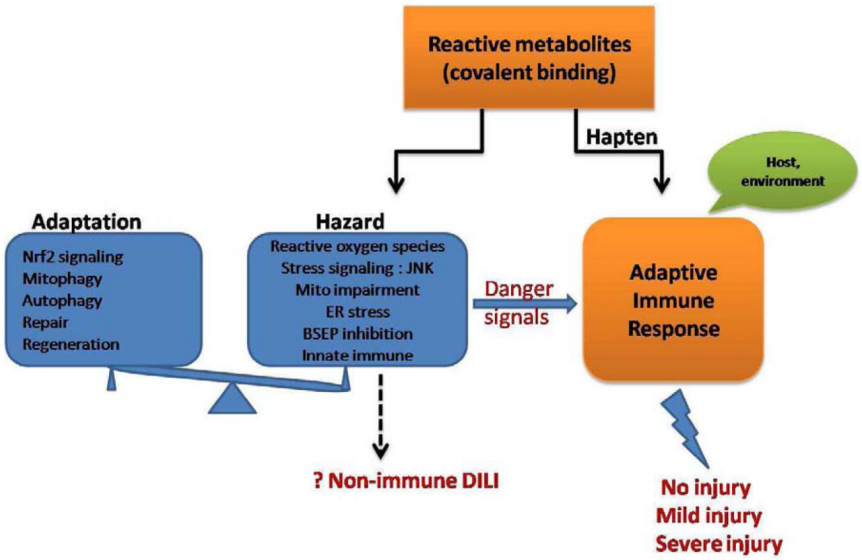

Fig. 2.

Hypothetical mechanisms of idiosyncratic drug-induced liver injury (IDILI). Adaptive immune response plays a major role in IDILI. This may be to some extent regulated by cellular events induced by drug and reactive metabolites. Specifically, drug or reactive metabolites may generate hazards in hepatocytes by inducing endoplasmic reticulum (ER) stress, generating ROS, activating stress signaling, impairing mitochondrial function, etc. To defend against these hazards, hepatocytes respond through adaptive mechanisms including nuclear factor erythroid 2-related factor 2 (Nrf-2) signaling, mitophagy and autophagy to cope with stress. The innate and adaptive immune systems can adapt to dampen the response. The ultimate outcome of liver is no injury, mild injury or severe injury. Although non-immune IDILI remains a theoretical possibility as a consequence of the hazards of reactive metabolites, aside from a small number of drugs which directly damage mitochondria, there are very few, if any, proven examples of this possibility.