HHS Public Access

Author manuscript

Liver Int. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Liver Int. 2018 August ; 38(8): 1363–1374. doi:10.1111/liv.13866.

Role and Mechanisms of Autophagy in Acetaminophen-induced Liver Injury

Xiaojuan Chao[1], **Hua Wang**[1,2], **Hartmut Jaeschke**[1], and **Wen-Xing Ding**[1,*]

[1]Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160, USA

[2]Department of Toxicology, School of Public Health, Anhui Medical University, Hefei, China

Abstract

Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure in the United States and many other countries. Although the metabolism and pathogenesis of APAP has been extensively investigated for decades, the mechanisms by which APAP induces liver injury are incompletely known, which hampers the development of effective therapeutic approaches to tackle this important clinical problem. Autophagy is a highly conserved intracellular degradation pathway, which aims at recycling cellular components and damaged organelles in response to adverse environmental conditions and stresses as a survival mechanism. There is accumulating evidence indicating that autophagy is activated in response to APAP overdose in specific liver zone areas, and pharmacological activation of autophagy protects against APAP-induced liver injury. Increasing evidence also suggests that hepatic autophagy is impaired in nonalcoholic fatty livers (NAFLD), and NAFLD patients are more susceptible to APAP-induced liver injury. Here we summarized the current progress on the role and mechanisms of autophagy in protecting against APAP-induced liver injury.

Keywords

autophagy; acetaminophen; mitophagy; acetaminophen protein adducts; liver injury

Introduction

Acetaminophen (APAP) is one of the most widely used antipyretic and analgesic drugs in the United States (US). It is reported to be regularly consumed by over 60 million Americans on a weekly basis (1). Though it is safe at therapeutic doses, an overdose can cause severe liver injury and even acute liver failure (ALF) in experimental animals and in humans. The United States Food and Drug Administration (FDA) recommended dose of APAP for adults is 650 to 1,000 mg every 4 to 6 hours, not to exceed 4g (possibly to 3,250

*Correspondence to: Wen-Xing Ding, Ph.D., Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, MS 1018, 3901 Rainbow Blvd., Kansas City, Kansas 66160, Fax: 913- 588-7501, wxding@kumc.edu.
DR. HARTMUT JAESCHKE (Orcid ID : 0000-0002-8695-6980)
DR. WEN-XING DING (Orcid ID : 0000-0002-3167-5073)

Conflicts of interest: The authors declare no conflict of interest.

mg) every 24 hours. In children, the dose is 10 to 15 mg/kg orally or rectally every 4 to 6 hours, up to 5 doses (or 50 to 75 mg/kg) in 24 hours. Consumption at this dose generally does not yield any toxic effects, and toxicity develops at 7.5 to 10 g/day, or 140 mg/kg (2). Due to the ubiquitous nature and broad availability of the drug, APAP overdose has been the most frequent cause of ALF in the US and other western countries, the second most common cause of liver transplantation worldwide and the most common in the US. Overall, APAP overdose is responsible for 56,000–80,000 emergency department visits, 26,000–34,000 hospitalizations, and an estimated 500 deaths per year in the US (3–6).

APAP metabolism and hepatotoxicity

The mechanisms that underlying APAP-induced liver injury have been extensively studied (7–11). At therapeutic doses, the majority (approximate 90%) of APAP is mainly metabolized via Phase-II reactions (glucuronidation and sulfation) and excreted into urine via the kidneys. The rest, approximately 10% is further metabolized by cytochrome P450 enzymes, predominately cytochrome P450 2E1 (CYP2E1) and CYP1A2, to form a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI). The highly reactive NAPQI further rapidly conjugates with hepatic glutathione (GSH) and is excreted into the bile without obvious harmful effects on hepatocytes. However, following overdose of APAP, both glucuronidation and sulfation pathways are saturated, and excessive NAPQI generation depletes intracellular and mitochondrial GSH within the liver. The remaining NAPQI then reacts and forms covalent links with cellular biological macromolecules, especially proteins, resulting in mitochondrial damages and necrotic cell death. When NAPQI links with proteins via their cysteine residues, APAP-protein adducts (APAP-AD) are formed. Mitochondrial proteins are significant targets of NAPQI, and APAP binds more frequently to mitochondrial proteins when compared to the non-hepatotoxic regioisomer 3′-hydroxyacetanilide (AMAP) in mouse liver (12). However, in human hepatocytes, AMAP can be hepatotoxic and its hepatotoxicity correlated with formation of mitochondrial protein adducts and compromised mitochondrial function (13). Subsequently, Bax (a pro-apoptotic Bcl-2 family protein) translocates to mitochondria to trigger the release of mitochondrial intermembrane proteins such as apoptosis-inducing factor (AIF) and endonuclease G to further induce nuclear DNA fragmentation. However, Bax seems to be important only for the early phase of APAP-induced necrosis (14). c-jun N-terminal kinase (JNK) and phosphorylated JNK are also recruited to mitochondria by SH3 homology associated BTK binding protein (Sab, or SH3BP5), an outer mitochondrial membrane protein, which further potentiates mitochondrial damage and necrosis (15). However, it remains to be determined whether formation of mitochondrial adducts plays a causal role in APAP-induced Bax and JNK mitochondrial translocation (14). Furthermore, specific targets in the mitochondria, including glutathione peroxidase (GPx) and the alpha subunit of adenosine triphosphate (ATP) synthase, are identified as APAP-adducts using proteomic analysis (16). However, it also remains unclear whether these mitochondrial adducts would directly impair mitochondrial functions and contribute to hepatocyte necrosis. It should be noted that the above mentioned factors such as Bcl-2 family proteins and JNK activation are also important mediators for apoptosis. The lack of apoptosis in wild type mouse livers after APAP is generally thought due to the severe mitochondrial damage and depletion of ATP, which

prevents the activation of caspases whose activation often relies on cellular ATP. The lack of relevance of apoptosis in APAP-induced hepatotoxicity is further supported by the evidence that the pan-caspase inhibitor did not improve APAP-induced liver injury (17). It should be noted that a small amount of apoptosis was observed in certain genetic knockout (KO) mouse strains such as syndecan-1, liver specific Atg7 or Atg5 knockout mice (18–20). The apoptosis observed in these KO mice could be due to the secondary effects as a result of the deletion of the gene in the liver such as the activation of Nrf2 in liver-specific Atg5 KO mice (19). Therefore it should be cautious to interpret data obtained from gene KO mice in APAP-induced liver injury. Consistent with the mouse studies, neither the active form of caspase 3 protein nor an increase in caspase 3 activity could be detected in samples from APAP overdose patients, even though full length caspase 3 was present (21). Collectively current evidence does not support a relevance of apoptosis in APAP-induced liver injury.

While there is compelling evidence to suggest that APAP induces hepatocyte necrosis, some recent findings support that receptor interacting protein (RIP)-mediated necroptosis may also be involved in APAP-induced liver injury despite it is still controversial (22–25). Necroptosis is regulated by RIP1-RIP3-MLKL (mixed lineage kinase domain-like protein)-mediated necrotic cascade (22, 26–28). RIP1 and RIP3 interacts with each other via their RIP homotypic interaction motif (RHIM) to form an amyloid-like structure termed the necrosome. The necrosome is stabilized by phosphorylated RIP1 and RIP3. Activated RIP3 then recruits and phosphorylates downstream MLKL protein. Phosphorylated MLKL translocates and oligomerizes at plasma membranes resulting in membrane rupture and necrosis (29, 30). We demonstrated that pharmacological inhibition of Drp1 (a mitochondrial fission protein) or RIP1 delayed APAP-induced cell death in primary cultured hepatocytes. RIP3 KO mice have decreased early phase APAP-induced liver injury (24). However, in another study, no protection against APAP-induced liver injury was observed in RIP3 and MLKL KO mice but instead only knockdown of RIP1 using an antisense approach protected against APAP-induced liver injury (31). Mechanistically, knockdown of RIP1 decreased mitochondrial translocation of JNK and Drp1 (31). However, liver-specific RIP1 KO mice, generated by crossing RIP1 flox/flox mice with albumin cre mice, did not show protection against APAP-induced liver injury (23). It is likely that the mouse strain and techniques used to manipulate RIP1/3 may account for these controversial findings. For example, C57bl/6J mice were used as wild type controls for RIP3 KO mice in Ramachandran et al studies(24), whereas C57bl/6N mice were used as wild type controls for RIP3 KO mice in Dara et al studies (31). The relevance of necroptosis in APAP-induced hepatocyte injury remains to be further elucidated.

Autophagy

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved degradation pathways for cytosolic macromolecules and damaged/excess organelles. Autophagy starts from the formation of a transient double-membrane structure, the phagophore. Following the expansion and closure, the phagophore becomes a complete autophagosome. Autophagosomes then fuse with lysosomes to form autolysosomes where the enwrapped contents are degraded by lysosomal enzymes (32–34). In mammalian cells, the initiation of autophagy requires the formation of the Unc-51 like autophagy activating

kinase 1 or 2 (ULK1 or ULK2, hereafter we only refer to ULK1) kinase complex at the phagophore assembly site (PAS) to allow the recruitment and activation of other Atg proteins. The ULK1 kinase complex is the most upstream component of the core autophagy machinery that is composed of ULK1, Atg13, RB1CC1/FIP200 (RB1-inducible coiled-coil 1) and C12orf44/Atg101 (35). ULK1 interacts with Atg13, which directly binds to FIP200 and mediates the interaction between FIP200 and ULK1. The activity of ULK1 kinase complex is regulated by both mechanistic target of rapamycin complex 1 (mTORC1) and the AMP-activated protein kinase (AMPK). When nutrients are sufficient, mTORC1 is activated and negatively regulates autophagy by directly phosphorylating ULK1 (S757) and inhibiting its activity (36, 37). In contrast, when cells suffer from insufficient energy, AMPK is activated and phosphorylates ULK1 at different sites (S317, S467, S555, T574, S637 and S777) and activates ULK1 to promote autophagy (38–40). Once activated, ULK1 further phosphorylates Beclin1 on S14 (41). Beclin1 is a component of the autophagy-promoting class III phosphoinositide 3-kinase (PtdIns3K) complexes, which also contains Atg14L, p150 and the class-III PI3 kinase VPS34 (42). The PtdIns3K complex is responsible for the production of phosphatidylinositol-3-phosphate (PtdIns3P) directly from phosphatidylinositol (43). This PtdIns3P serves as a landmark on the membrane to recruit other factors, such as Atg18, involved in the process of autophagosome formation (44). After the activation of PtdIns3K complex, phagophore expands and elongates by membrane addition, which is accomplished by two essential ubiquitin-like (Ubl) conjugation systems, which involve the Ubl proteins Atg12 and microtubule-associated protein light chain 3 (LC3) (45). In the ubiquitin system, Atg12 is first activated by an E1-like enzyme, Atg7. Subsequently, Atg12 is transferred to an E2-like conjugating enzyme, Atg10, and further binds to Atg16L1 to form the Atg12-Atg5-Atg16L1 complex. Nascent LC3 is first processed by a protease, Atg4, to expose its C terminus glycine, which is called LC3-I form that resides in the cytosol. LC3-I is then further activated by E1-like enzyme, Atg7, then transferred to an E2-like conjugating enzyme, Atg3. Finally, LC3 conjugates with PE to form a membrane associated LC3-II form, with the Atg12-Atg5-Atg16L1 complex participating as an E3-like ligase. In addition, Atg9-mediated cycling systems containing the core protein Atg9, Atg2 and WIPI1/2 contributes to the elongation of the phagophore (46). Atg9 is thought to participate in membrane delivery from donor sources, such as the trans-Golgi network or late endosomes, to the expanding phagophore. The movement of Atg9 is dependent on the activity of the ULK1, PtdIns3K, and mitogen-activated protein kinase 14 (MAPK4/p38) (47). Eventually, the expanding membrane closes around its cargo to form a complete autophagosome. Autophagosomes move along microtubules in a dynein motor-dependent manner and cluster closed to the microtubule-organizing center near the nucleus, where they fuse with lysosomes to form autolysosomes. The mechanism that controls the timing of fusion remains unknown. UVRAG, which is part of the PtdIns3K complex, activates the GTPase Rab7, which promotes fusion with lysosomes (48, 49). Other components of the SNARE (soluble NSF [N-ethyl-maleimide-sensitive fusion protein] attachment protein receptor) family proteins, such as VAMP7, VAMP8, VAMP9 and STX17/syntaxin 17, are also implicated in fusion of autophagosomes with lysosomes (50–52). LAMP-2, a lysosomal membrane protein, is also required for the fusion of autophagosomes with lysosomes. Lack of LAMP-2 prevents the localization of STX17 and SNAP-29 on the autophagosomes resulting in defective fusion of autophagosomes with lysosomes (53). After

the sequestered cargo is delivered inside the lysosome, it is broken down by resident hydrolases of the lysosome and the resulting macromolecules are released back into the cytosol as new energy sources and building blocks for cell survival (Figure 1). In addition to providing new building blocks and energy sources, autophagy also helps to remove damaged organelles such as mitochondria as another important mechanism for cell survival.

Autophagy in APAP-induced liver injury

Since damaged mitochondria and ATP depletion are critical factors for APAP-induced hepatocyte necrosis, it is reasonable to hypothesize that autophagy may be an important protective mechanism against APAP-induced liver injury because autophagy can help to remove damaged mitochondria and also provide energy fuel for ATP production. Using GFP-LC3 transgenic mice, we demonstrate that APAP administration increased the number of GFP-LC3 positive autophagosomes in the liver pericentral areas (54). Further careful electron microscopy analysis, we further defined an unique zonated pattern of biochemical and pathological changes in APAP-treated mouse livers, including necrosis (zone 1), mitochondrial spheroid formation (zone 2), autophagy (zone 3) and mitochondrial biogenesis (zone 4) (55). Increased autophagosomes often occur adjacent to the necrotic areas, which perhaps act as a defense barrier to restrict the expanding of the necrosis to the relatively normal liver zone areas. Increased autophagy protects against APAP-induced liver injury was subsequently confirmed by several other groups (56–58). For instance, administration of adiponectin protects against APAP-induced hepatotoxicity by activating AMPK and ULK11-mediated autophagy (56). Krüppel-like factor 6 (KLF6), a transcription factor and tumor suppressor, has been shown to transcriptionally up-regulate Atg7 and Beclin1 and protects against APAP-induced liver injury (57). Acid sphingomyelinase (ASMase) KO mice have increased lysosomal cholesterol accumulation and impaired autophagy, which are more susceptible to APAP-induced liver injury (58).

Mechanistically, APAP inhibits mTOR activity in primary hepatocytes (54). APAP may inhibit mTORC1 activity through increased generation of mitochondrial ROS and decreased cellular ATP levels, leading to AMPK-ULK1 activation and autophagy induction. Pharmacological induction of autophagy by rapamycin or torin1 protects against, while inhibition of autophagy by 3-Methyladenine (3-MA), chloroquine (CQ) or leupeptin further exacerbates APAP-induced necrosis and liver injury (54, 59). However, results from genetic autophagy-deficient mouse models yield conflicting results for APAP-induced liver injury likely due to compensatory effects in these autophagy-deficient mouse livers (19, 20, 60). Nevertheless, emerging evidence indicates that autophagic removal of damaged mitochondria (via mitophagy) and APAP-AD play critical roles in protecting against APAP-induced liver injury, which is discussed in detail below.

Mitophagy

One of the key mechanisms to timely removal of damaged mitochondria is a specific form of autophagy termed as mitophagy. Among the signaling pathways that are responsible for mitophagy, phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1)/Parkin pathway is so far the best characterized. In healthy mitochondria, PINK1, a mitochondrial

serine/threonine kinase, is imported through the outer membrane via the translocase of mitochondrial membrane (TOM) complex and the inner mitochondrial membrane via the TIM complex. PINK1 then is first processed by the matrix processing peptidase (61) and cleaved by the inner mitochondrial membrane protease PARL (62–64). This new truncated form of PINK1 is released into the cytosol for N-end recognition and degraded by the proteasome (65). When mitochondria are damaged and depolarized, PINK1 is no longer imported into the inner membrane or cleaved by PARL resulting in its accumulation on the outer mitochondrial membrane (66–68). PINK1 then phosphorylates Ser65 on ubiquitin and Ser65 within Parkin's UBL domain that leads to the recruitment and tethering of Parkin to mitochondria. PINK1-mediated Ser65 phosphorylation of ubiquitin also markedly enhances Parkin E3 ligase activity resulting in greater Parkin-induced outer mitochondrial membrane protein ubiquitination (69). Parkin ubiquitinates a variety of outer mitochondrial membrane proteins including the mitochondrial fusion proteins mitofusin 1 (Mfn1) and Mfn2, the mitochondrial trafficking protein Miro1, the TOM20, and the voltage-dependent anion channel (VDAC). Degradation of Mfn1/Mfn2 causes mitochondrial fragmentation, which may further facilitate mitophagy since smaller mitochondria are likely more readily to be engulfed by autophagosomes (70–73). Ubiquitination and degradation of Miro1 leads to mitochondrial arrest, which can separate damaged mitochondria from healthy mitochondria to facilitate the engulfment of damaged mitochondria by autophagosomes (74).

Several receptor proteins including SQSTM1/p62, optineurin, NDP52, Nix, Bnip3, Fundc1 and prohibitin 2 have been reported to act specifically for mitophagy in a Parkin-dependent or independent manner (75–78). These mitophagy receptor proteins may be redundant and can compensate for each other. Therefore, loss of one receptor protein may not necessarily lead to a severe mitophagy defect. In addition, the importance of each of these receptor proteins in mitophagy may also be context- and cell type-dependent (79, 80).

Interestingly, mitophagy can also be induced via Parkin-independent pathways. Parkin-independent mitophagy may be mediated by other E3 ubiquitin ligases such as Smurf1, March5, or Mule. Parkin-independent mitophagy may also occur via Bcl2/adenovirus E1B 19 kDa interacting protein 3 (Bnip3), Fun 14 domain containing 1 (Fundc1), Nix, Bcl2L13, cardiolipin, or Ambra1 (81). In addition, PINK1 can directly recruit NDP52 and optineurin to mitochondria independent of Parkin (80). PINK1 also activates tank binding kinase 1 (TBK1), which then phosphorylates adaptor proteins optineurin, NDP52, and p62 on depolarized mitochondria. Phosphorylated optineurin and p62 have enhanced binding affinity to LC3 and thus enhances recruitment of autophagosomes to mitochondria (79). However, in the absence of Parkin, PINK1 only leads to the induction of a low level of mitophagy. In the presence of Parkin, PINK1-generated phospho-ubiquitin serves as the autophagy signal on mitochondria that further brings Parkin to mitochondria to trigger robust and rapid mitophagy induction (69).

We recently demonstrated that APAP administration increases Parkin translocation to mitochondria with concurrent increased ubiquitination of mitochondrial proteins and mitophagy induction in mouse livers (82). Unexpectedly, we found that Parkin KO mice are resistant to APAP-induced liver injury (81). Several possible mechanisms may help to explain these contradictory findings. First, mitophagy still occurs in Parkin KO mouse livers

although to a lesser extent after APAP administration compared with wild type mice. These results imply that PINK1-mediated mitophagy may occur in the Parkin KO mouse livers after APAP treatment. Indeed, we recently generated PINK1/Parkin double KO mice and we found that mitophagy markedly decreased in PINK1/Parkin double KO mouse livers after APAP treatment (83). More importantly, PINK1/Parkin double KO mice were more susceptible to APAP-induced liver injury (83). Second, it is possible that some mitophagy receptor proteins such as SQSTM1/p62, Nix, Bnip3 and Fundc1 may directly recruit the autophagy machinery to mitochondria independent of PINK1 and Parkin. However, none of these receptor proteins have yet been examined in APAP-induced mitophagy. Third, other ubiquitin E3 ligases such as March5, Mule and Smurf1 could also be involved in APAP-induced mitochondrial protein ubiquitination and mitophagy, which yet remains to be investigated. Fourth, APAP-induced dysfunctional mitochondria may also be removed via formation of an autophagosome-like structure that is derived from damaged mitochondria, which we termed as “mitochondrial spheroids” (84, 85). However, whether mitochondrial spheroids may degrade mitochondria themselves remains unknown. Mitochondrial spheroids only occur in the pericentral zone area adjacent to the necrotic areas in APAP-treated mouse livers (55), which may serve as an alternative mechanism to remove APAP-induced damaged mitochondria. Fifth, Parkin KO mice have decreased JNK activation but have increased myeloid leukemia cell differentiation protein (Mcl-1) expression as well as increased hepatocyte proliferation in their livers after APAP treatment. All these compensatory effects are blunted by acute knocking down of Parkin in mouse livers. As a result, mice with acute knockdown of Parkin in the livers become more susceptible to APAP-induced liver injury. These results not only emphasize the important role of Parkin in APAP-induced mitophagy and liver injury but also raise concerns on the interpretation of data using the chronic deletion and acute knockdown of Parkin in APAP-induced liver injury in mice. Taken together, despite of the evidence supporting a role of mitophagy in protecting against APAP-induced liver injury, future works are needed to identify the mitophagy receptor proteins and potential Parkin-independent pathways in APAP-induced mitophagy and liver injury.

Removal of APAP-adducts by autophagy

As discussed above, APAP can form APAP-AD in hepatocytes in both mice and humans (86). APAP-AD has also been detected in mitochondria and may contribute to APAP-induced mitochondrial dysfunction and subsequent oxidant stress (13, 16). Therefore, timely removal of APAP-AD may help to attenuate APAP-induced mitochondrial damage to maintain normal mitochondrial function and provide sufficient ATP. These are critical processes necessary for the recovery of mice and humans from APAP intoxication (87, 88). Despite the importance of APAP-AD formation, the mechanisms by which hepatocytes remove APAP-AD has been largely unknown. We recently provided compelling evidence to show that autophagy helps to remove APAP-AD in hepatocytes. First, APAP-AD displayed a punctate peri-nuclear pattern in hepatocytes, which are co-localized with GFP-LC3 puncta and lysosome-associated membrane protein 1 (Lamp1), an autophagosome and a lysosome marker, respectively (59). Second, purified hepatic autophagosomes and autolysosomes contained APAP-AD, and the levels of autophagosome-enwrapped APAP-AD increased in the presence of the autophagy inhibitor CQ. Third, pharmacological inhibition of autophagy

by leupeptin increased the serum levels of APAP-AD. Fourth, leupeptin dramatically increased the levels of detergent insoluble APAP-AD. Since detergent insoluble protein aggregates are mainly cleared by autophagy, these findings further support a role of autophagy in the removal of APAP-AD. Finally, APAP-AD are associated with the autophagy receptor protein p62 and knockdown of p62 leads to impaired clearance of APAP-AD and increase APAP-induced hepatotoxicity (59). Taken together, these findings suggest that autophagy may protect against APAP-induced liver injury by removing APAP-AD.

Autophagy regulates GSH biosynthesis and JNK activation

As discussed above, despites its highly reactive nature, NAPQI can be detoxified by conjugating with GSH. It has been well documented that the covalent binding between NAPQI and cellular proteins is highly correlated with APAP-induced cytotoxicity. Therefore, the regulation of the intracellular GSH levels via its biosynthesis is a critical factor for APAP-induced hepatotoxicity. Because of this, N-acetylcysteine (NAC), a precursor of GSH, has been the primary antidote for APAP overdose for several decades. The first step of GSH synthesis involves the formation of γ-glutamylcysteine from glutamate and cysteine catalyzed by glutamate-cysteine ligase (GCL, also known as γ-glutamylcysteine synthetase), which is the rate-determining step of the GSH synthesis pathway. The second step involves the addition of glycine to γ-glutamylcysteine to yield GSH catalyzed by GSH synthetase (GS).

Nnuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) is a transcription factor responsible for regulation of gene expression of GSH biosynthesis and other antioxidant genes, which plays a critical role in protecting against APAP-induced liver injury (89, 90). Nrf2 can be activated via the “canonical” and “non-canonical” pathway. For canonical Nrf2 activation, under normal conditions, Keap1 (Kelch-like ECH-associated protein 1) recruits a Cullin 3-Ring Box Protein 1 E3 ubiquitin ligase complex to Nrf2 resulting in the ubiquitination and proteasomal degradation of Nrf2 (91, 92). In response to oxidative/electrophilic stress, the cysteine residue (C151) of Keap1 is oxidized resulting in the disruption of Keap1and Nrf2 interaction and subsequent stabilization of Nrf2. Nrf2 then enters nuclei to heterodimerize with small musculoaponeurotic fibrosarcoma (sMAF) proteins and binds to the antioxidant response element (ARE) of target genes to activate their transcription (91).

Increasing evidence indicates that Nrf2 can also be activated by the non-canonical pathway through SQSTM1/p62, an autophagy substrate protein that generally accumulates in cells with impaired autophagy (19, 93, 94). p62 directly interacts with Keap1 and in turn releases Nrf2 from Keap1 resulting in Nrf2 accumulation and activation (95, 96). p62 itself is a target gene of Nrf2 (97), implying that a positive feedback loop exists within the p62/Keap1/Nrf2 axis. Moreover, serine 349, located at KIR (keap1 interacting region) of p62, is phosphorylated under stress conditions by mTORC1, and phosphorylated p62 has higher affinity for Keap1 resulting in higher Nrf2 activation (98). Since autophagy generally degrades p62, inhibition of autophagy would lead to the accumulation of p62 and subsequent Nrf2 activation. Indeed, liver-specific Atg5 KO have increased hepatic p62 protein and

persistent Nrf2 activation (19, 94). The mRNA and protein levels of GCLC, GCLM and NQO1, are all upregulated in liver-specific Atg5 KO mice. As a result, these mice also have higher basal GSH levels and faster recovery of hepatic GSH after its depletion by NAPQI and are resistant to APAP-induced liver injury (19). These results indicate that activation of the p62-Keap1-Nrf2 axis plays a protective role against APAP-induced liver injury.

Contrary to our report, it was reported by Igusa et al that APAP-induced liver injury was enhanced in liver specific Atg7 KO mice, accompanying with increased cleavage of caspase 3/7, ROS production, mitochondrial membrane depolarization, and JNK activation (20). In contrast to use albumin cre mice, Igusa Y et al injected polyinosinic acid-polycytidylic acid (pIpC) to eliminate Atg7 in mouse livers. pIpC may trigger immune response and also affect P450 enzyme activities (60), which may explain why increased apoptosis was observed after APAP treatment in these Atg7 KO mice. As discussed above, APAP mainly induces hepatocyte necrosis but not apoptosis in both mice and humans. Therefore, the relevance of these findings from Atg7 KO mice by Igusa et al is questionable (60).

More recently, Sun et al found that ULK1/ULK2 double KO mice are also resistant to APAP-induced liver injury. Surprisingly, ULK1/ULK2 double KO mice have intact hepatic autophagy and therefore no p62-mediated Nrf2 activation was found in ULK1/ULK2 double KO mice (99). Instead, ULK1 is required for the maximum activation hepatic JNK in response to APAP treatment. Mechanistically, APAP inhibits mTORC1 resulting in the release of activated ULK1, which then directly phosphorylates and increases the kinase activity of mitogen-activated protein kinase kinase 4 and 7 (MKK4/7), the upstream kinases and activator of JNK. Liver-specific ULK1/ULK2 double KO mice have impaird APAP-induced JNK activation and are thus resistant to APAP-induced liver injury (99). Taken together, it seems that chronic inhibition of autophagy may lead to compensatory non-canonical activation of Nrf2 and protects against APAP-induced liver injury. In addition, some autophagy proteins such as ULK1 may regulate JNK activation and APAP-induced liver injury independent of its autophagy function.

Autophagy and nonalcoholic fatty liver disease in APAP hepatotoxicity

Obesity and nonalcoholic fatty liver disease (NAFLD) is an increasing health problem worldwide. NAFLD is characterized as the accumulation of lipid droplets in hepatocytes and is a leading cause of chronic liver disease in US and other western countries (100). The prevalence of NAFLD has been estimated to occur in more than 20% of adults in general and more than 30% in US (100). NAFLD can further progress to nonalcoholic steatohepatitis (NASH) with increased inflammation, hepatocyte death and ballooning as well as fibrosis, ultimately leading to cirrhosis and liver cancer. Hepatic autophagy is impaired in both genetic and diet-induced obese mice, which is associated with endoplasmic reticulum stress and down-regulated autophagy proteins (101). In obese human livers and diet-induced obese mouse livers, *S*-nitrosoglutathione reductase (GSNOR), a major protein denitrosylase, is impaired resulting in increased *S*-nitrosylation of lysosomal proteins in the liver and impaired autophagic-lysosomal degradation (102). Overexpression of autophagy proteins or pharmacological activation of autophagy improves insulin resistance and hepatic steatosis in diet-induced NAFLD (101–103).

Results from both clinical human and experimental animal studies generally suggests that NAFLD may promote APAP-induced liver injury although some conflicting observations also exist (104). Several clinical studies revealed that NAFLD patients may have a higher risk of APAP-induced hepatotoxicity but not obesity itself (105, 106). However, experimental evidence from rodents yields mixed conclusions. While rats fed with a high fat diet are more susceptible to APAP-induced liver injury, obese Zucker fa/fa rats that have the leptin receptor gene mutation are resistant to APAP-induced liver injury (107, 108). Female db/db mice (with leptin receptor gene mutation) are also more susceptible to APAP-induced liver injury whereas female ob/ob mice (with leptin gene mutation) show the same sensitivity to APAP-induced liver injury (109). Induction of hepatic Cyp2E1 has been frequently observed in NAFLD, which may be linked to the increased APAP hepatotoxicity. However, not all studies mentioned above determined the activity of hepatic Cyp2E1 except one study reported that Cyp2E1 activity increased in female db/db but not in ob/ob mice (109), which seems to correlate with their sensitivity to APAP-induced liver injury. The use of female mice and rats in these studies could also be a problem because it is known that rats and female mice are generally resistant to APAP-induced liver injury (110, 111). In addition, the possible role of autophagy in NAFLD and APAP-induced liver injury has not been studied in these studies. Therefore, it will be interesting to study whether improve impaired hepatic autophagy in NAFLD by pharmacological autophagy activators would attenuate APAP-induced liver injury in the future.

Autophagy and sterile inflammation in APAP hepatotoxicity

After an APAP overdose, cell necrosis can lead to release of local damage-associated molecular patterns (DAMPs) including mitochondrial DNA (21), nuclear DNA fragments (112), high mobility group box 1 (HMGB1) (113, 114), uric acid (115), adenosine triphosphate (ATP) (116), and many other cellular constituents (117). These DAMPs cause the transcriptional activation of inflammatory cytokines in macrophages through Toll-like receptors (TLRs) and inflammasome activation (118–120). Stimulation of TLR9 by DNA fragments during APAP-induced cell death can lead to the transcriptional activation of the IL-1β gene resulting in the formation of pro-IL-1β (121). The pro-form of IL-1β is then cleaved by activated caspase-1 to yield the active cytokine (122). Caspase-1 activation is regulated by the assembly of the inflammasome, which consists of NLRP3 (NACHT, LRR, and pyrin domain-containing protein 3), ASC (apoptosis-associated speck-like protein containing a CARD), and Pro-caspase-1 (123, 124). This NLRP3 inflammasome can be activated by a wide range of stimuli that include both pathogenic microorganisms and endogenous mediators, such as ROS, mitochondrial DAMPs and ATP, crystalline structures (eg, uric acid) and other fibrillar proteins (eg, β-amyloid fibrils) and environmental irritants (eg, silica, alum) (124, 125). It has been generally hypothesized that sterile inflammation and perhaps inflammasome activation may contribute to a second phase of injury during APAP intoxication (11, 120). However, the role of inflammation in APAP-induced liver injury is still controversial and has been extensively reviewed recently (11, 120).

Saitoh *et al.* reported that autophagy can negatively regulate inflammasome activation (126). Deletion of Atg16L1 leads to increased caspase-1 activation and production of IL-1β and IL-18 in macrophages after LPS stimulation. Treatment with autophagy inhibitor 3-MA or

genetically inhibition of autophagy (Atg7, LC3B or Beclin1 deficiency), significantly enhanced IL-1β production in response to inflammasome inducers (127, 128). In contrast, TLR- or rapamycin-induced autophagy leads to reduced amount of pro-IL-1β (129). The regulation of inflammasome activation by autophagy can occur in multiple ways, through either removal of endogenous inflammasome activators or removal of inflammasomes and their downstream cytokines directly. Autophagy directly sequesters and degrades ubiquitinated-inflammasomes and pro-IL-1β molecules or indirectly lowers endogenous sources that induce inflammasome formation, such as mitochondrial ROS and DNA (130). Autophagy selectively removes APAP-induced damaged mitochondrial by mitophagy and decreases ROS production in hepatocytes, which might subsequently inhibit inflammasome activation. However, the contribution of autophagy-mediated inflammasome inactivation in APAP-induced liver injury remains to be studied.

Clinical Relevance of autophagy in acetaminophen-induced liver injury in humans

Accumulating evidence now indicates that the key hepatotoxic mechanisms and signaling pathways discovered in mice are also true in APAP overdosed humans. While direct evidence from APAP overdosed human livers/biopsies is not available, experimental data from primary cultured human hepatocytes and metabolic competent HepaRG cells support the notion that APAP triggers hepatotoxicity via similar mechanisms in human cells and mice. For example, the formation of APAP protein adducts, depletion of GSH, mitochondrial damage and JNK activation that observed in mouse livers and primary mouse hepatocytes are also reproducible in human hepatocytes (13, 131, 132). We also found that APAP increased autophagic flux in primary cultured human hepatocytes (59). Moreover, pharmacological inhibition of autophagy increased levels of APAP protein adducts and APAP-induced necrosis in human hepatocytes (59). These data may imply that the protective effects of pharmacological activation of autophagy against APAP-induced liver injury in mice may also be true in humans. Clinical trial studies using pharmacological autophagy activators such as mTOR inhibitor Torin 1 in APAP overdose patients will be an exciting future direction.

In addition to studies using primary cultured hepatocytes, significant progress has also been made in identifying serum biomarkers in APAP-induced liver injury in mice and humans (133). Interestingly, we found that the serum levels of LC3-II, the specific form of LC3 that is associated with autophagosomal membrane formation, is elevated in APAP-treated mice and APAP overdosed humans (59). More importantly, serum LC3-II was detected not only in APAP overdose patients with high serum levels of ALT and APAP protein adducts but also in patients with low ALT levels. Our observation suggests that the level of serum LC3-II may be a more sensitive biomarker than ALT and APAP protein adducts in predicting the severity of liver injury induced by APAP. One limitation of the use of serum LC3-II is that it may not be specific for APAP-induced liver injury because LC3-II can also be detected in endotoxin-induced liver injury in mice. However, if combined with the serum APAP protein adducts, serum levels of LC3-II may serve as a novel mechanistic biomarker and have a potential values for predicting the course and severity of APAP-induced liver injury in

humans. More work is definitely needed to further explore the clinical beneficial effects of activation of autophagy and evaluate other serum autophagy markers in addition to LC3-II in APAP overdose patients.

Conclusion and future perspective

In summary, increasing evidence supports an important role of autophagy in protecting against APAP-induced liver injury by selectively removal APAP-AD and damaged mitochondria. While data from the genetic autophagy-deficient mice for APAP-induced liver injury are complex due to the compensatory effects in mouse livers such as Nrf2 activation and JNK inhibition, pharmacological upregulation of autophagy may be a potential therapeutic option for treatment of APAP-induced liver injury. Future studies are needed to dissect the role of autophagy in APAP-induced liver injury in NAFLD patients. Future studies are also needed to identify pharmacological approaches to specifically targeting PINK1-Pakin-mediated mitophagy. The molecular events that regulate autophagy and mitophagy and their roles in APAP-induced liver injury are summarized in Figure 2.

Acknowledgments

Financial support: This work was partially supported by NIH grants: R01 AA020518, R01 DK102142, U01 AA024733 and P20GM103549 & P30GM118247, and CSC (No. 201708040024).

List of Abbreviations

APAP	acetaminophen
ALF	acute liver failure
CYP2E1	cytochrome P450 2E1
NAPQI	*N*-acetyl-*p*-benzoquinone imine
GSH	glutathione
APAP-DA	APAP-protein adducts
AMAP	3′-hydroxyacetanilide
AIF	apoptosis-inducing factor
JNK	c-jun N-terminal kinase
SH3BP5/Sab	SH3 homology associated BTK binding protein
GPx	glutathione peroxidase
ATP	adenosine triphosphate
RIP	receptor interacting protein
MLKL	mixed lineage kinase domain-like protein
RHIM	RIP homotypic interaction motif

ULK1/2	Unc-51 like autophagy activating kinase 1/2
Atg	autophagy-related gene
PAS	phagophore assembly site
RB1CC1/FIP200	RB1-inducible coiled-coil 1
mTORC1	mechanistic target of rapamycin complex 1
AMPK	AMP-activated protein kinase
PtdIns3P	phosphoinositide 3-kinase; phosphatidylinositol-3-phosphate
Ubl	ubiquitin-like
LC3/MAP1-LC3	microtubule-associated protein 1A/1B light chain 3
PE	phosphatidylethanolamine
MAPK4/p38	mitogen-activated protein kinase 14
SNARE	soluble NSF [N-ethyl-maleimide-sensitive fusion protein] attachment protein receptor
KLF6	Krüppel-like factor 6
ASMase	Acid sphingomyelinase
ROS	reactive oxygen species
3-MA	3-Methyladenine
CQ	chloroquine
MPTP	mitochondrial membrane permeability transition pore
PINK1	phosphatase and tensin homolog (PTEN)-induced kinase 1
MTS	mitochondrial targeting sequence
TOM	translocase of mitochondrial membrane
Mfn1	mitofusin 1
VDAC	voltage-dependent anion channel
Bnip3	Bcl2/adenovirus E1B 19 kDa interacting protein 3
Fundc1	Fun 14 domain containing 1
TBK1	tank binding kinase 1
KO	knockout
Mcl-1	myeloid leukemia cell differentiation protein

APAP-Cys	APAP-cysteine
Lamp1	lysosome-associated membrane protein 1
NAC	N-acetylcysteine
GCL	glutamate-cysteine ligase
GS	GSH synthetase
Nrf2/NFE2L2	nuclear factor (erythroid-derived 2)-like 2
Keap1	Kelch-like ECH-associated protein 1
sMAF	small musculoaponeurotic fibrosarcoma
ARE	antioxidant response element
GCLC	GCL catalytic subunit
GCLM	GCL modifier subunit
KIR	keap1 interacting region
pIpC	polyinosinic acid-polycytidylic acid
MKK4/7	mitogen-activated protein kinase kinase 4 and 7
NAFLD	nonalcoholic fatty liver disease
GSNOR	*S*-nitrosoglutathione reductase
DAMPs	damage-associated molecular patterns
HMGB1	high-mobility group box 1
TLRs	Toll-like receptors
NLRP3	NACHT, LRR, and pyrin domain-containing protein 3
ASC	apoptosis-associated speck-like protein containing a CARD

References

1. HERNDON CM, DANKENBRING DM. Patient perception and knowledge of acetaminophen in a large family medicine service. J Pain Palliat Care Pharmacother. 2014; 28(2):109–16. [PubMed: 24813653]
2. CLARK R, FISHER JE, SKETRIS IS, JOHNSTON GM. Population prevalence of high dose paracetamol in dispensed paracetamol/opioid prescription combinations: an observational study. BMC Clin Pharmacol. 2012; 12:11. [PubMed: 22709372]
3. ALTYAR A, KORDI L, SKREPNEK G. Clinical and economic characteristics of emergency department visits due to acetaminophen toxicity in the USA. BMJ Open. 2015; 5(9):e007368.
4. NOURJAH P, AHMAD SR, KARWOSKI C, WILLY M. Estimates of acetaminophen (Paracetomal)-associated overdoses in the United States. Pharmacoepidemiol Drug Saf. 2006; 15(6):398–405. [PubMed: 16294364]

5. MANTHRIPRAGADA AD, ZHOU EH, BUDNITZ DS, LOVEGROVE MC, WILLY ME. Characterization of acetaminophen overdose-related emergency department visits and hospitalizations in the United States. Pharmacoepidemiol Drug Saf. 2011; 20(8):819–26. [PubMed: 21294217]
6. BUDNITZ DS, LOVEGROVE MC, CROSBY AE. Emergency department visits for overdoses of acetaminophen-containing products. Am J Prev Med. 2011; 40(6):585–92. [PubMed: 21565648]
7. LARSON AM. Acetaminophen hepatotoxicity. Clin Liver Dis. 2007; 11(3):525–48. vi. [PubMed: 17723918]
8. MCGILL MR, JAESCHKE H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharm Res. 2013; 30(9):2174–87. [PubMed: 23462933]
9. NELSON SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin Liver Dis. 1990; 10(4):267–78. [PubMed: 2281334]
10. HINSON JA, ROBERTS DW, JAMES LP. Mechanisms of acetaminophen-induced liver necrosis. Handb Exp Pharmacol. 2010; (196):369–405.
11. RAMACHANDRAN A, JAESCHKE H. Acetaminophen Toxicity Novel Insights into Mechanisms and Future Perspectives. Gene Expr. 2017
12. TIRMENSTEIN MA, NELSON SD. Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a nonhepatotoxic regioisomer, 3′-hydroxyacetanilide, in mouse liver. The Journal of biological chemistry. 1989; 264(17):9814–9. [PubMed: 2524496]
13. XIE Y, MCGILL MR, DU K, et al. Mitochondrial protein adducts formation and mitochondrial dysfunction during N-acetyl-m-aminophenol (AMAP)-induced hepatotoxicity in primary human hepatocytes. Toxicology and applied pharmacology. 2015; 289(2):213–22. [PubMed: 26431796]
14. BAJT ML, FARHOOD A, LEMASTERS JJ, JAESCHKE H. Mitochondrial bax translocation accelerates DNA fragmentation and cell necrosis in a murine model of acetaminophen hepatotoxicity. The Journal of pharmacology and experimental therapeutics. 2008; 324(1):8–14. [PubMed: 17906064]
15. WIN S, THAN TA, MIN RW, AGHAJAN M, KAPLOWITZ N. c-Jun N-terminal kinase mediates mouse liver injury through a novel Sab (SH3BP5)-dependent pathway leading to inactivation of intramitochondrial Src. Hepatology. 2016; 63(6):1987–2003. [PubMed: 26845758]
16. QIU Y, BENET LZ, BURLINGAME AL. Identification of the hepatic protein targets of reactive metabolites of acetaminophen in vivo in mice using two-dimensional gel electrophoresis and mass spectrometry. The Journal of biological chemistry. 1998; 273(28):17940–53. [PubMed: 9651401]
17. GUJRAL JS, KNIGHT TR, FARHOOD A, BAJT ML, JAESCHKE H. Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? Toxicological sciences : an official journal of the Society of Toxicology. 2002; 67(2):322–8. [PubMed: 12011492]
18. NAM EJ, HAYASHIDA K, AQUINO RS, et al. Syndecan-1 limits the progression of liver injury and promotes liver repair in acetaminophen-induced liver injury in mice. Hepatology. 2017; 66(5): 1601–15. [PubMed: 28543100]
19. NI HM, BOGGESS N, MCGILL MR, et al. Liver-specific loss of Atg5 causes persistent activation of Nrf2 and protects against acetaminophen-induced liver injury. Toxicological sciences : an official journal of the Society of Toxicology. 2012; 127(2):438–50. [PubMed: 22491424]
20. IGUSA Y, YAMASHINA S, IZUMI K, et al. Loss of autophagy promotes murine acetaminophen hepatotoxicity. Journal of gastroenterology. 2012; 47(4):433–43. [PubMed: 22124574]
21. MCGILL MR, SHARPE MR, WILLIAMS CD, TAHA M, CURRY SC, JAESCHKE H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. The Journal of clinical investigation. 2012; 122(4):1574–83. [PubMed: 22378043]
22. DEGTEREV A, HUANG Z, BOYCE M, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nature chemical biology. 2005; 1(2):112–9. [PubMed: 16408008]
23. SCHNEIDER AT, GAUTHERON J, TACKE F, VUCUR M, LUEDDE T. Receptor interacting protein kinase 1 (RIPK1) in hepatocytes does not mediate murine acetaminophen toxicity. Hepatology. 2016; 64(1):306–8.

24. RAMACHANDRAN A, MCGILL MR, XIE Y, NI HM, DING WX, JAESCHKE H. Receptor interacting protein kinase 3 is a critical early mediator of acetaminophen-induced hepatocyte necrosis in mice. Hepatology. 2013; 58(6):2099–108. [PubMed: 23744808]
25. YANG X, CHAO X, WANG ZT, DING WX. The end of RIPK1-RIPK3-MLKL-mediated necroptosis in acetaminophen-induced hepatotoxicity? Hepatology. 2016; 64(1):311–2. [PubMed: 26418225]
26. ZHANG DW, SHAO J, LIN J, et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science. 2009; 325(5938):332–6. [PubMed: 19498109]
27. CHO YS, CHALLA S, MOQUIN D, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell. 2009; 137(6): 1112–23. [PubMed: 19524513]
28. HE S, WANG L, MIAO L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell. 2009; 137(6):1100–11. [PubMed: 19524512]
29. WEINLICH R, GREEN DR. The two faces of receptor interacting protein kinase-1. Molecular cell. 2014; 56(4):469–80. [PubMed: 25459879]
30. VANDEN BERGHE T, LINKERMANN A, JOUAN-LANHOUET S, WALCZAK H, VANDENABEELE P. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nature reviews Molecular cell biology. 2014; 15(2):135–47. [PubMed: 24452471]
31. DARA L, JOHNSON H, SUDA J, et al. Receptor interacting protein kinase 1 mediates murine acetaminophen toxicity independent of the necrosome and not through necroptosis. Hepatology. 2015; 62(6):1847–57. [PubMed: 26077809]
32. NAKATOGAWA H, SUZUKI K, KAMADA Y, OHSUMI Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nature reviews Molecular cell biology. 2009; 10(7):458–67. [PubMed: 19491929]
33. PARZYCH KR, KLIONSKY DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxidants & redox signaling. 2014; 20(3):460–73. [PubMed: 23725295]
34. KLIONSKY DJ, EMR SD. Autophagy as a regulated pathway of cellular degradation. Science. 2000; 290(5497):1717–21. [PubMed: 11099404]
35. MIZUSHIMA N. The role of the Atg1/ULK1 complex in autophagy regulation. Curr Opin Cell Biol. 2010; 22(2):132–9. [PubMed: 20056399]
36. EGAN D, KIM J, SHAW RJ, GUAN KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy. 2011; 7(6):643–4. [PubMed: 21460621]
37. KIM J, GUAN KL. Regulation of the autophagy initiating kinase ULK1 by nutrients: roles of mTORC1 and AMPK. Cell Cycle. 2011; 10(9):1337–8. [PubMed: 21403467]
38. EGAN DF, SHACKELFORD DB, MIHAYLOVA MM, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science. 2011; 331(6016):456–61. [PubMed: 21205641]
39. KIM J, KUNDU M, VIOLLET B, GUAN KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011; 13(2):132–41. [PubMed: 21258367]
40. LOFFLER AS, ALERS S, DIETERLE AM, et al. Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. Autophagy. 2011; 7(7):696–706. [PubMed: 21460634]
41. RUSSELL RC, TIAN Y, YUAN H, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol. 2013; 15(7):741–50. [PubMed: 23685627]
42. KIHARA A, NODA T, ISHIHARA N, OHSUMI Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae. The Journal of cell biology. 2001; 152(3):519–30. [PubMed: 11157979]
43. BURMAN C, KTISTAKIS NT. Regulation of autophagy by phosphatidylinositol 3-phosphate. FEBS Lett. 2010; 584(7):1302–12. [PubMed: 20074568]
44. OBARA K, SEKITO T, NIIMI K, OHSUMI Y. The Atg18-Atg2 complex is recruited to autophagic membranes via phosphatidylinositol 3-phosphate and exerts an essential function. J Biol Chem. 2008; 283(35):23972–80. [PubMed: 18586673]

45. OHSUMI Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nature reviews Molecular cell biology. 2001; 2(3):211–6. [PubMed: 11265251]

46. POLSON HE, DE LARTIGUE J, RIGDEN DJ, et al. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy. 2010; 6(4):506–22. [PubMed: 20505359]

47. PAPINSKI D, SCHUSCHNIG M, REITER W, et al. Early steps in autophagy depend on direct phosphorylation of Atg9 by the Atg1 kinase. Mol Cell. 2014; 53(3):471–83. [PubMed: 24440502]

48. JAGER S, BUCCI C, TANIDA I, et al. Role for Rab7 in maturation of late autophagic vacuoles. J Cell Sci. 2004; 117(Pt 20):4837–48. [PubMed: 15340014]

49. LIANG C, LEE JS, INN KS, et al. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nat Cell Biol. 2008; 10(7):776–87. [PubMed: 18552835]

50. FADER CM, SANCHEZ DG, MESTRE MB, COLOMBO MI. TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. Biochim Biophys Acta. 2009; 1793(12):1901–16. [PubMed: 19781582]

51. FURUTA N, FUJITA N, NODA T, YOSHIMORI T, AMANO A. Combinational soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins VAMP8 and Vti1b mediate fusion of antimicrobial and canonical autophagosomes with lysosomes. Mol Biol Cell. 2010; 21(6):1001–10. [PubMed: 20089838]

52. ITAKURA E, KISHI-ITAKURA C, MIZUSHIMA N. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell. 2012; 151(6): 1256–69. [PubMed: 23217709]

53. HUBERT V, PESCHEL A, LANGER B, GROGER M, REES A, KAIN R. LAMP-2 is required for incorporating syntaxin-17 into autophagosomes and for their fusion with lysosomes. Biol Open. 2016; 5(10):1516–29. [PubMed: 27628032]

54. NI HM, BOCKUS A, BOGGESS N, JAESCHKE H, DING WX. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatology. 2012; 55(1):222–32. [PubMed: 21932416]

55. NI HM, WILLIAMS JA, JAESCHKE H, DING WX. Zonated induction of autophagy and mitochondrial spheroids limits acetaminophen-induced necrosis in the liver. Redox biology. 2013; 1:427–32. [PubMed: 24191236]

56. LIN Z, WU F, LIN S, et al. Adiponectin protects against acetaminophen-induced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice. Journal of hepatology. 2014; 61(4):825–31. [PubMed: 24882054]

57. SYDOR S, MANKA P, BEST J, et al. Kruppel-like factor 6 is a transcriptional activator of autophagy in acute liver injury. Scientific reports. 2017; 7(1):8119. [PubMed: 28808340]

58. BAULIES A, RIBAS V, NUNEZ S, et al. Lysosomal Cholesterol Accumulation Sensitizes To Acetaminophen Hepatotoxicity by Impairing Mitophagy. Scientific reports. 2015; 5:18017. [PubMed: 26657973]

59. NI HM, MCGILL MR, CHAO X, et al. Removal of acetaminophen protein adducts by autophagy protects against acetaminophen-induced liver injury in mice. Journal of hepatology. 2016; 65(2): 354–62. [PubMed: 27151180]

60. JAESCHKE H, DING WX. Autophagy and acetaminophen hepatotoxicity: how useful are Atg7-deficient mice? Journal of gastroenterology. 2012; 47(7):845–6. author reply 47–8. [PubMed: 22565638]

61. GREENE AW, GRENIER K, AGUILETA MA, et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. EMBO reports. 2012; 13(4):378–85. [PubMed: 22354088]

62. JIN SM, LAZAROU M, WANG C, KANE LA, NARENDRA DP, YOULE RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. The Journal of cell biology. 2010; 191(5):933–42. [PubMed: 21115803]

63. DEAS E, PLUN-FAVREAU H, GANDHI S, et al. PINK1 cleavage at position A103 by the mitochondrial protease PARL. Human molecular genetics. 2011; 20(5):867–79. [PubMed: 21138942]

64. MEISSNER C, LORENZ H, WEIHOFEN A, SELKOE DJ, LEMBERG MK. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. Journal of neurochemistry. 2011; 117(5):856–67. [PubMed: 21426348]

65. YAMANO K, YOULE RJ. PINK1 is degraded through the N-end rule pathway. Autophagy. 2013; 9(11):1758–69. [PubMed: 24121706]

66. NARENDRA DP, JIN SM, TANAKA A, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. 2010; 8(1):e1000298. [PubMed: 20126261]

67. MATSUDA N, SATO S, SHIBA K, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol. 2010; 189(2):211–21. [PubMed: 20404107]

68. VIVES-BAUZA C, ZHOU C, HUANG Y, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A. 2010; 107(1):378–83. [PubMed: 19966284]

69. KOYANO F, OKATSU K, KOSAKO H, et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature. 2014; 510(7503):162–6. [PubMed: 24784582]

70. CHAN NC, SALAZAR AM, PHAM AH, et al. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Human molecular genetics. 2011; 20(9):1726–37. [PubMed: 21296869]

71. GEGG ME, COOPER JM, CHAU KY, ROJO M, SCHAPIRA AH, TAANMAN JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Human molecular genetics. 2010; 19(24):4861–70. [PubMed: 20871098]

72. GEISLER S, HOLMSTROM KM, SKUJAT D, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nature cell biology. 2010; 12(2):119–31. [PubMed: 20098416]

73. THOMAS KJ, MCCOY MK, BLACKINTON J, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Human molecular genetics. 2011; 20(1):40–50. [PubMed: 20940149]

74. WANG X, WINTER D, ASHRAFI G, et al. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. Cell. 2011; 147(4):893–906. [PubMed: 22078885]

75. WILLIAMS JA, DING WX. Mechanisms, pathophysiological roles and methods for analyzing mitophagy - recent insights. Biological chemistry. 2018; 399(2):147–78. [PubMed: 28976892]

76. WEI YJ, CHIANG WC, SUMPTER R, MISHRA P, LEVINE B. Prohibitin 2 Is an Inner Mitochondrial Membrane Mitophagy Receptor. Cell. 2017; 168(1–2):224. [PubMed: 28017329]

77. LAZAROU M, SLITER DA, KANE LA, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2015; 524(7565):309. [PubMed: 26266977]

78. ZHONG ZY, UMEMURA A, SANCHEZ-LOPEZ E, et al. NF-kappa B Restricts Inflammasome Activation via Elimination of Damaged Mitochondria. Cell. 2016; 164(5):896–910. [PubMed: 26919428]

79. HEO JM, ORDUREAU A, PAULO JA, RINEHART J, HARPER JW. The PINK1-PARKIN Mitochondrial Ubiquitylation Pathway Drives a Program of OPTN/NDP52 Recruitment and TBK1 Activation to Promote Mitophagy. Molecular cell. 2015; 60(1):7–20. [PubMed: 26365381]

80. LAZAROU M, SLITER DA, KANE LA, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2015; 524(7565):309–14. [PubMed: 26266977]

81. WILLIAMS JA, DING WX. Mechanisms, pathophysiological roles and methods for analyzing mitophagy - recent insights. Biological chemistry. 2018; 399(2):147–78. [PubMed: 28976892]

82. WILLIAMS JA, NI HM, HAYNES A, et al. Chronic Deletion and Acute Knockdown of Parkin Have Differential Responses to Acetaminophen-induced Mitophagy and Liver Injury in Mice. The Journal of biological chemistry. 2015; 290(17):10934–46. [PubMed: 25752611]

83. WANG H, NI HM, JAESCHKE H, DING WX. Double Deletion of Pink1 and Parkin Eliminates Compensatory Effects of a Single deletion of Pink1 or Parkin on Acetaminophen-Induced Mitophagy and Liver Injury. Hepatology. 2017:417A.

84. DING WX, LI M, BIAZIK JM, et al. Electron microscopic analysis of a spherical mitochondrial structure. The Journal of biological chemistry. 2012; 287(50):42373–8. [PubMed: 23093403]

85. DING WX, GUO F, NI HM, et al. Parkin and mitofusins reciprocally regulate mitophagy and mitochondrial spheroid formation. The Journal of biological chemistry. 2012; 287(50):42379–88. [PubMed: 23095748]

86. JAMES LP, ALONSO EM, HYNAN LS, et al. Detection of acetaminophen protein adducts in children with acute liver failure of indeterminate cause. Pediatrics. 2006; 118(3):e676–81. [PubMed: 16950959]

87. MCGILL MR, LEBOFSKY M, NORRIS HR, et al. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. Toxicol Appl Pharmacol. 2013; 269(3):240–9. [PubMed: 23571099]

88. HEARD KJ, GREEN JL, JAMES LP, et al. Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. BMC Gastroenterol. 2011; 11:20. [PubMed: 21401949]

89. ENOMOTO A, ITOH K, NAGAYOSHI E, et al. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicological sciences : an official journal of the Society of Toxicology. 2001; 59(1):169–77. [PubMed: 11134556]

90. OKAWA H, MOTOHASHI H, KOBAYASHI A, ABURATANI H, KENSLER TW, YAMAMOTO M. Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochemical and biophysical research communications. 2006; 339(1): 79–88. [PubMed: 16293230]

91. ZHANG DD. The Nrf2-Keap1-ARE signaling pathway: The regulation and dual function of Nrf2 in cancer. Antioxidants & redox signaling. 2010; 13(11):1623–6. [PubMed: 20486759]

92. HARDER B, JIANG T, WU T, et al. Molecular mechanisms of Nrf2 regulation and how these influence chemical modulation for disease intervention. Biochemical Society transactions. 2015; 43(4):680–6. [PubMed: 26551712]

93. BJORKOY G, LAMARK T, BRECH A, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol. 2005; 171(4): 603–14. [PubMed: 16286508]

94. NI HM, WOOLBRIGHT BL, WILLIAMS J, et al. Nrf2 promotes the development of fibrosis and tumorigenesis in mice with defective hepatic autophagy. Journal of hepatology. 2014; 61(3):617–25. [PubMed: 24815875]

95. KOMATSU M, KUROKAWA H, WAGURI S, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nature cell biology. 2010; 12(3):213–23. [PubMed: 20173742]

96. LAU A, WANG XJ, ZHAO F, et al. A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. Molecular and cellular biology. 2010; 30(13):3275–85. [PubMed: 20421418]

97. JAIN A, LAMARK T, SJOTTEM E, et al. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. The Journal of biological chemistry. 2010; 285(29):22576–91. [PubMed: 20452972]

98. ICHIMURA Y, WAGURI S, SOU YS, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell. 2013; 51(5):618–31. [PubMed: 24011591]

99. SUN Y, LI TY, SONG L, et al. Liver-specific deficiency of unc-51 like kinase 1 and 2 protects mice from acetaminophen-induced liver injury. Hepatology. 2017

100. BRUNT EM, WONG VWS, NOBILI V, et al. Nonalcoholic fatty liver disease. Nat Rev Dis Primers. 2015:1.

101. YANG L, LI P, FU S, CALAY ES, HOTAMISLIGIL GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell metabolism. 2010; 11(6):467–78. [PubMed: 20519119]

102. QIAN Q, ZHANG Z, ORWIG A, et al. S-Nitrosoglutathione Reductase Dysfunction Contributes to Obesity-Associated Hepatic Insulin Resistance via Regulating Autophagy. Diabetes. 2018; 67(2):193–207. [PubMed: 29074597]

103. SETTEMBRE C, DE CEGLI R, MANSUETO G, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. Nature cell biology. 2013; 15(6):647–58. [PubMed: 23604321]

104. MICHAUT A, MOREAU C, ROBIN MA, FROMENTY B. Acetaminophen-induced liver injury in obesity and nonalcoholic fatty liver disease. Liver Int. 2014; 34(7):e171–9. [PubMed: 24575957]

105. NGUYEN GC, SAM J, THULUVATH PJ. Hepatitis C is a predictor of acute liver injury among hospitalizations for acetaminophen overdose in the United States: a nationwide analysis. Hepatology. 2008; 48(4):1336–41. [PubMed: 18821593]

106. MYERS RP, SHAHEEN AA. Hepatitis C, alcohol abuse, and unintentional overdoses are risk factors for acetaminophen-related hepatotoxicity. Hepatology. 2009; 49(4):1399–400. [PubMed: 19330871]

107. CORCORAN GB, WONG BK. Obesity as a risk factor in drug-induced organ injury: increased liver and kidney damage by acetaminophen in the obese overfed rat. The Journal of pharmacology and experimental therapeutics. 1987; 241(3):921–7. [PubMed: 3598908]

108. BLOUIN RA, DICKSON P, MCNAMARAPJ, CIBULL M, MCCLAIN C. Phenobarbital induction and acetaminophen hepatotoxicity: resistance in the obese Zucker rodent. The Journal of pharmacology and experimental therapeutics. 1987; 243(2):565–70. [PubMed: 3681696]

109. AUBERT J, BEGRICHE K, DELANNOY M, et al. Differences in early acetaminophen hepatotoxicity between obese ob/ob and db/db mice. The Journal of pharmacology and experimental therapeutics. 2012; 342(3):676–87. [PubMed: 22647274]

110. DU K, WILLIAMS CD, MCGILL MR, JAESCHKE H. Lower susceptibility of female mice to acetaminophen hepatotoxicity: Role of mitochondrial glutathione, oxidant stress and c-jun N-terminal kinase. Toxicology and applied pharmacology. 2014; 281(1):58–66. [PubMed: 25218290]

111. MCGILL MR, WILLIAMS CD, XIE Y, RAMACHANDRAN A, JAESCHKE H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicology and applied pharmacology. 2012; 264(3):387–94. [PubMed: 22980195]

112. MARQUES PE, OLIVEIRA AG, PEREIRA RV, et al. Hepatic DNA deposition drives drug-induced liver injury and inflammation in mice. Hepatology. 2015; 61(1):348–60. [PubMed: 24824608]

113. ANTOINE DJ, WILLIAMS DP, KIPAR A, et al. High-mobility group box-1 protein and keratin-18, circulating serum proteins informative of acetaminophen-induced necrosis and apoptosis in vivo. Toxicol Sci. 2009; 112(2):521–31. [PubMed: 19783637]

114. MARTIN-MURPHY BV, HOLT MP, JU C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. Toxicol Lett. 2010; 192(3):387–94. [PubMed: 19931603]

115. KONO H, CHEN CJ, ONTIVEROS F, ROCK KL. Uric acid promotes an acute inflammatory response to sterile cell death in mice. J Clin Invest. 2010; 120(6):1939–49. [PubMed: 20501947]

116. HOQUE R, SOHAIL MA, SALHANICK S, et al. P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol Gastrointest Liver Physiol. 2012; 302(10):G1171–9. [PubMed: 22383490]

117. LIU ZX, HAN D, GUNAWAN B, KAPLOWITZ N. Neutrophil depletion protects against murine acetaminophen hepatotoxicity. Hepatology. 2006; 43(6):1220–30. [PubMed: 16729305]

118. KUBES P, MEHAL WZ. Sterile inflammation in the liver. Gastroenterology. 2012; 143(5):1158–72. [PubMed: 22982943]

119. WILLIAMS CD, ANTOINE DJ, SHAW PJ, et al. Role of the Nalp3 inflammasome in acetaminophen-induced sterile inflammation and liver injury. Toxicol Appl Pharmacol. 2011; 252(3):289–97. [PubMed: 21396389]

120. WOOLBRIGHT BL, JAESCHKE H. Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure. J Hepatol. 2017; 66(4):836–48. [PubMed: 27913221]

121. IMAEDA AB, WATANABE A, SOHAIL MA, et al. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. J Clin Invest. 2009; 119(2):305–14. [PubMed: 19164858]

122. SIMS JE, SMITH DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010; 10(2): 89–102. [PubMed: 20081871]

123. KANNEGANTI TD, LAMKANFI M, NUNEZ G. Intracellular NOD-like receptors in host defense and disease. Immunity. 2007; 27(4):549–59. [PubMed: 17967410]

124. GUO H, CALLAWAY JB, TING JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med. 2015; 21(7):677–87. [PubMed: 26121197]

125. DAVIS BK, WEN H, TING JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol. 2011; 29:707–35. [PubMed: 21219188]

126. SAITOH T, FUJITA N, JANG MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2008; 456(7219):264–8. [PubMed: 18849965]

127. SHI CS, SHENDEROV K, HUANG NN, et al. Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol. 2012; 13(3):255–63. [PubMed: 22286270]

128. NAKAHIRA K, HASPEL JA, RATHINAM VA, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol. 2011; 12(3):222–30. [PubMed: 21151103]

129. HARRIS J, HARTMAN M, ROCHE C, et al. Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. J Biol Chem. 2011; 286(11):9587–97. [PubMed: 21228274]

130. ZHOU R, YAZDI AS, MENU P, TSCHOPP J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011; 469(7329):221–5. [PubMed: 21124315]

131. XIE Y, MCGILL MR, COOK SF, et al. Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells. Xenobiotica; the fate of foreign compounds in biological systems. 2015; 45(10):921–9. [PubMed: 25869248]

132. MCGILL MR, YAN HM, RAMACHANDRAN A, MURRAY GJ, ROLLINS DE, JAESCHKE H. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology. 2011; 53(3):974–82. [PubMed: 21319200]

133. MCGILL MR, JAESCHKE H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. Expert Opin Drug Metab Toxicol. 2014; 10(7):1005–17. [PubMed: 24836926]

Key Points

- Acetaminophen (APAP) overdose has become one of the most common cause of acute liver failure and intentional or accidental death in many counties.
- Increasing evidences indicated that APAP overdose activated autophagy as a protective process against APAP-induced liver injury.
- Patients with nonalcoholic fatty liver disease may be more susceptible to APAP-induced liver injury via impaired hepatic autophagy.
- The current progress on the role and mechanisms of autophagy in protecting against APAP-induced liver injury was summarized in this review.

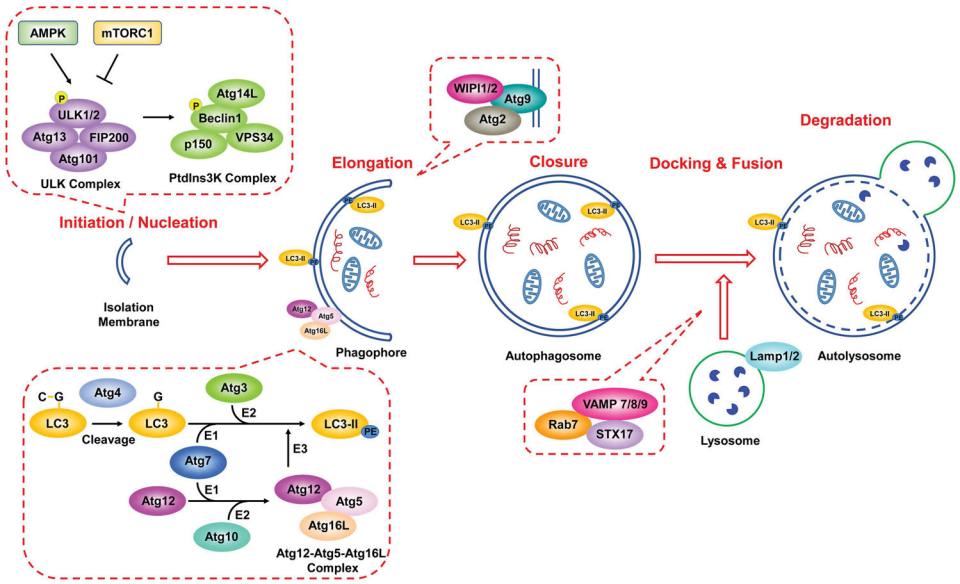

Figure 1. A simplified scheme for the regulation of autophagy in mammalian cells

When nutrients are sufficient, mTORC1 is activated and phosphorylates ULK1/2 and inhibits its activity. In contrast, when cells suffer from insufficient energy, AMPK is activated and phosphorylates ULK1/2 to activate ULK1/2. The active ULK1/2 phosphorylates Beclin-1, leading to VPS34 activation and phagophore formation. ULK1/2 functions in a complex with FIP200, Atg13 and Atg101, whereas VPS34 function requires a regulatory subunit, p150 and Beclin-1. Nascent LC3 is first processed by a protease, Atg4, to expose its C terminus glycine to form LC3-I form. LC3-I is then further activated by E1-like enzyme, Atg7, then transferred to an E2-like conjugating enzyme, Atg3. Finally, LC3 conjugates with PE to form a membrane associated LC3-II form, with the Atg12-Atg5-Atg16L1 complex participating as an E3-like ligase. Atg9-mediated cycling systems containing the core protein Atg9, Atg2 and WIPI1/2 contributes to the elongation of the phagophore by delivering membrane from donor sources. The closure of an elongated phagophore marks the formation of a mature autophagosome, which eventually fuses with a lysosome, leading to cargo degradation. GTPase Rab7 and some components of the SNARE family proteins, such as VAMP7, VAMP8, VAMP9 and STX17, and lysosomal-associated membrane protein 1/2 are implicated in autophagosome fusion with lysosomes.

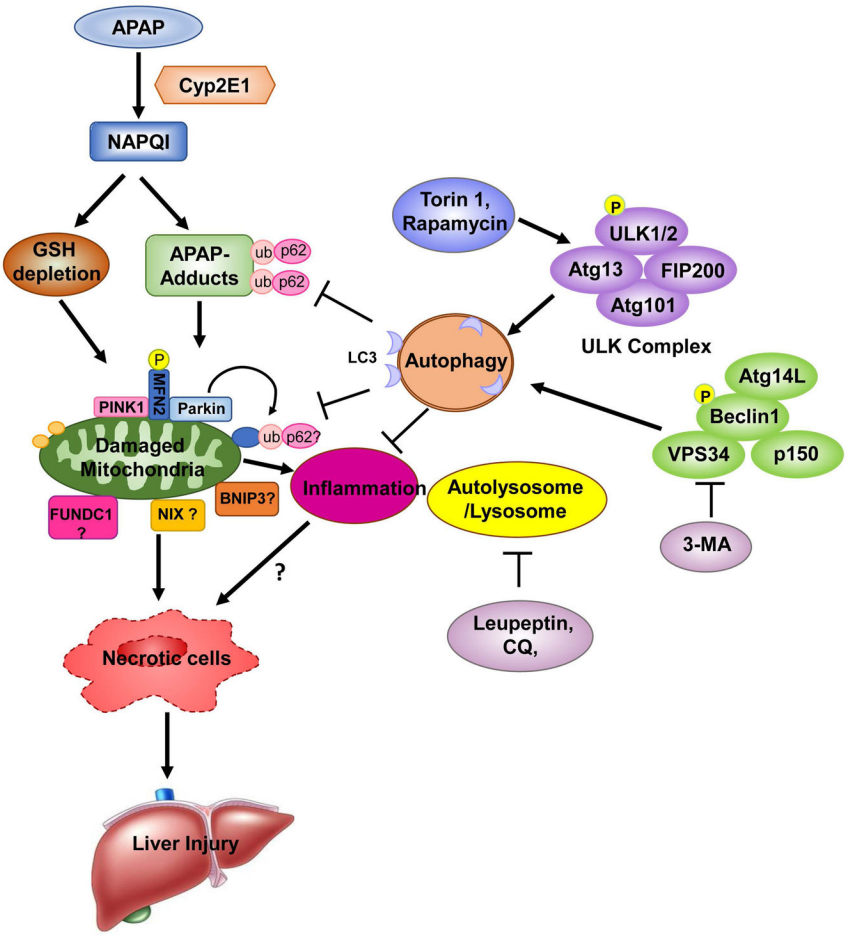

Figure 2. A proposed model for the mechanisms underlying the protection of autophagy against acetaminophen hepatotoxicity

APAP is first metabolized by cytochrome P450 enzymes (mainly via Cyp2E1) to generate the reactive metabolite NAPQI, which depletes hepatic GSH and binds to cellular and mitochondrial proteins to form APAP-Adducts (APAP-AD) resulting in a mitochondrial oxidant stress. The increasing oxidative stress and mitochondrial protein adducts trigger mitochondrial damage. Damaged mitochondria can lead to necrotic cell death, inflammation and subsequent liver injury. p62, an autophagy receptor protein, is recruited to APAP-AD, which may facilitate APAP-AD transition to the detergent insoluble form and allow their recognition and sequestration by autophagosomes and eventual removal by autolysosomes. Damaged mitochondria could be removed through PINK1-Parkin mediated selective mitophagy. Damaged mitochondria can stabilize mitochondrial PINK1 that recruits Parkin to mitochondria to trigger the ubiquitination of mitochondrial proteins. The mitochondrial ubiquitination-p62-LC3 complex promotes the recognition and removal of damaged mitochondria by mitophagy although the role of p62 in the APAP context has not been determined. In addition to PINK1/Parkin pathway, several other mitochondrial outer membrane proteins (FUNDC1, Bnip3 and Nix) may directly interact with LC3 through their LC3 interacting region to mediate mitophagy independent of PINK1 and Parkin, but their roles in APAP-induced mitophagy have not been studied. Autophagy can suppress

inflammation and inflammasome activation by removing damage mitochondrial and mitochondrial DNA. Torin 1 or rapamycin can target the ULK1 complex to induce autophagy to protect against APAP-induced liver injury via removal of APAP-adducts and damaged mitochondria. In contrast, 3-MA inhibits VPS34 complex to inhibit the upstream autophagosome formation whereas Leupeptin or CQ impairs autolysosome/lysosome functions leading to the exacerbation of APAP-induced necrosis and liver injury, respectively.