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Review Article

Inflammasome Activation as a Key Driver of Acetaminophen-induced Hepatotoxicity: Mechanisms and Emerging Therapeutics

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Abstract

Acetaminophen (APAP) is one of the most commonly used analgesic and antipyretic medications and is generally considered safe at therapeutic doses. However, overdose remains a leading cause of acute liver failure, primarily characterized by centrilobular (zone 3) hepatic necrosis, oxidative stress, mitochondrial dysfunction, and sterile inflammation. The hepatotoxic effects of APAP are localized to the centrilobular region, where cytochrome P450 2E1 is highly expressed. Cytochrome P450 2E1 catalyzes the conversion of APAP to a toxic metabolite, N-acetyl-p-benzoquinone imine. During overdose, the liver's detoxification capacity is overwhelmed and excess N-acetyl-p-benzoquinone imine binds to cellular proteins, initiating oxidative stress and mitochondrial injury that culminate in hepatocyte death. A central component of APAP-induced hepatotoxicity is the activation of innate immune responses, particularly via inflammasome pathways. Inflammasomes are cytosolic multiprotein complexes that detect cellular damage and trigger inflammation. Among these, the NOD-, LRR-, and pyrin domain-containing 3 (NLRP3) inflammasome plays a significant role in APAP-induced liver injury. Upon activation, the NLRP3 inflammasome promotes autocatalytic cleavage of procaspase-1 into its active form, caspase-1, which subsequently processes the pro-inflammatory cytokines pro-interleukin-1β and pro-interleukin-18 into their mature forms. These cytokines recruit additional immune cells and amplify liver inflammation, exacerbating tissue injury. Thus, the NLRP3 inflammasome serves as a key mechanistic link between the initial toxic insult and the ensuing inflammatory response in APAP hepatotoxicity. This review aimed to explore the molecular mechanisms underlying APAP-induced liver injury, particularly inflammasome activation, and evaluate the current and emerging therapeutic strategies.

Introduction

Acetaminophen (APAP), commonly known as paracetamol, is a widely used over-the-counter medication renowned for its analgesic and antipyretic properties. Despite its ubiquity and recognized safety when taken within recommended doses, APAP overdose remains a significant public health concern worldwide due to liver injury, posing a substantial burden on healthcare systems and contributing to a considerable number of hospitalizations and fatalities annually.[1] APAP overdose frequently occurs due to various factors, including intentional or unintentional ingestion beyond recommended doses or accidental overdoses resulting from unawareness of the presence of APAP in various combination products. Furthermore, in individuals with a history of alcoholism, fasting, or liver disease, even therapeutic doses of APAP can cause liver injury.[2] The accessibility and widespread availability of APAP contribute to its high prevalence as a cause of acute liver injury. APAP toxicity, whether resulting from a single overdose or repeated supratherapeutic ingestion, progresses through four phases: preclinical toxic effects (phase one), hepatic injury (phase two), hepatic failure (phase three), and recovery (phase four). Early intervention during the preclinical phase generally leads to full recovery with minimal liver damage. However, delayed treatment after hepatic injury presents a variable prognosis, and progression to hepatic failure carries a mortality rate of 20% to 40%.[3]

The hepatotoxic effects of APAP overdose are well documented

Keywords: Acetaminophen; Caspase-1; Drug-induced liver injury; Inflammasome; NOD-, LRR-, and pyrin domain-containing 3; NLRP3.

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and primarily arise from the metabolic conversion of APAP to reactive metabolites in the liver. Under normal conditions, APAP is metabolized via conjugation with sulfate and glucuronide, leading to its excretion in the urine. However, a small fraction of APAP undergoes oxidative metabolism by cytochrome P450 enzymes, predominantly cytochrome P450 2E1 (CYP2E1), to form N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive and toxic intermediate. The subsequent detoxification of NAPQI occurs through its conjugation with glutathione (GSH), a crucial endogenous antioxidant in the liver. However, when APAP is ingested in excessive amounts, the capacity of GSH to neutralize NAPQI becomes overwhelmed, leading to depletion of hepatic GSH stores and accumulation of NAPQI, resulting in oxidative stress, mitochondrial dysfunction, and hepatocellular injury, ultimately culminating in acute liver injury and potential liver failure. The clinical manifestations of APAP overdose vary widely depending on the dose ingested, the time elapsed since ingestion, and individual patient factors.[2]

Early symptoms may include gastrointestinal disturbances such as nausea, vomiting, and abdominal pain, which may progress to more severe manifestations, including jaundice, hepatic encephalopathy, and coagulopathy in patients with advanced liver injury.[4] While timely recognition and intervention are crucial for mitigating the adverse effects of APAP overdose, effective treatment options are available to manage hepatotoxicity and prevent progression to liver failure. Despite treatment, hepatotoxicity occurs in 12–13% of acute APAP overdoses, with 2–5% of cases progressing to liver failure and 0.2–0.5% resulting in fatalities.[5,6] N-acetylcysteine (NAC), a precursor of GSH, serves as the cornerstone of therapy for APAP overdose by replenishing depleted GSH stores and enhancing the detoxification of NAPQI. The administration of NAC within the appropriate timeframe reduces the risk of hepatotoxicity and significantly improves patient outcomes.[7] Despite advancements in understanding the pathophysiology of APAP overdose and the development of effective treatment strategies, a considerable proportion of cases still progress to hepatotoxicity, liver failure, and even mortality. This review is significant as it provides a comprehensive exploration of the role of inflammasomes in APAP-induced liver injury, a critical but often underemphasized aspect of the pathophysiology of this condition. While the hepatotoxic effects of APAP overdose are well documented, the intricate interplay between oxidative stress, cellular damage, and the inflammatory response requires further elucidation. The purpose of this review was to critically evaluate the molecular basis of APAP-induced liver injury, with a focus on inflammasome signaling and emerging therapeutic interventions. By incorporating recent advances, this work provides a comprehensive understanding of the role of cytosolic multiprotein complexes, particularly inflammasomes, in the molecular pathogenesis of liver injury. Furthermore, this review identifies potential therapeutic targets by elucidating the mechanisms of inflammasome activation and their downstream effects. Such information is crucial for developing novel treatments aimed at mitigating the inflammatory response and reducing liver damage in patients with APAP-induced liver injury. Additionally, this study underscores the need for clinical approaches that address both the toxicological aspects and modulation of the inflammatory response to improve patient outcomes.

Mechanistic pathways associated with acetaminophen-induced hepatotoxicity

APAP is rapidly absorbed and extensively metabolized upon entering the liver, with 85–90% conjugated with glucuronic acid or sulfate by the enzymes uridine diphosphate-glucuronosyltransferase and sulfotransferase and subsequently eliminated. This conjugation renders APAP metabolites water-soluble, aiding their excretion via urine or bile. By converting APAP into water-soluble forms, this phase II pathway helps detoxify and eliminate APAP from the body, reducing the risk of metabolite accumulation and potential harm.[8] When APAP is administered at therapeutic doses under normal physiological conditions, the remaining 5–10% of the drug is metabolized in hepatocytes by cytochrome P450 enzymes such as CYP2E1 and CYP1A2 through phase I enzymatic reactions, resulting in the generation of the toxic reactive metabolite NAPQI (Fig. 1). Glutathione-S-transferase catalyzes the conjugation of NAPQI with GSH, forming APAP-GSH adducts,[9,10] which are further metabolized into nontoxic thiolate and cysteine molecules, including adducts of cysteamide, glycine/cysteine, and N-acetyl-L-cysteine. Phase III involves the transport of these metabolites by transporters, after which they are eliminated through bile or urine, preventing potential adverse effects on the liver.

However, following APAP toxicity, endogenous glucuronide and sulfate cofactors become depleted, leading to increased concentrations of NAPQI and rapid depletion of cellular GSH. Consequently, the accumulated NAPQI binds covalently to the cysteine, methionine, tyrosine, and tryptophan residues of various proteins, forming NAPQI-protein adducts within the cell that disrupt normal physiological protein functions.[11,12] NAPQI binds to housekeeping proteins, glutathione peroxidase (GPx),[13] and adenosine triphosphate (ATP) synthase,[14,15] among other proteins often abundant in mitochondria.[12] Free radicals are generated when NAPQI oxidizes thiol groups in proteins.[16] Furthermore, NAPQI binds to complexes I and II, disrupting the electron transport chain and allowing electrons to escape and produce superoxide radicals.[17] Complex II is more susceptible to NAPQI inhibition than complex I.[17] The formation of peroxynitrite occurs when superoxide radicals interact with nitric oxide, resulting in nitrotyrosine adducts within mitochondria. NAPQI binding modifies the α-subunit of mitochondrial ATP synthase, reducing ATP production.[14] Therefore, peroxynitrite and reactive oxygen species (ROS) damage mitochondrial DNA, impair cellular respiration and ATP synthesis, and ultimately cause mitochondrial dysfunction (Fig. 2). Mitochondrial enzymes, such as GPx, catalase, and manganese superoxide dismutase 2, frequently neutralize free radicals, including superoxide anions and hydrogen peroxide. Thioredoxin (Trx), present in the cytosol as Trx1 and in mitochondria as Trx2, is oxidized and upregulated in response to oxidative stress and elevated ROS levels.[18] The phosphorylation of apoptosis signal-regulating kinase 1 (ASK1) is triggered by the dissociation of ASK1 from Trx, resulting in ASK1 activation (Fig. 2). Activated ASK1 phosphorylates c-Jun N-terminal kinase (JNK) via cytosolic mitogen-activated protein kinase 4/7.[19] Following further translocation to the mitochondria, activated phosphorylated JNK initiates a cascade in which JNK binds to the outer mitochondrial membrane scaffold protein Sab, inhibiting electron transport and thereby increasing the generation of ROS and peroxynitrite.[20]

Furthermore, phosphorylated JNK induces oxidative stress, mitochondrial membrane permeability, and dysfunction through the activation of Bax, the translocation of Bax to the mitochondria, the opening of the mitochondrial permeability transition pore, and Src-dependent inhibition of the electron transport complex.[12,21,22] The opening of the mitochondrial permeability transition pore under pathological conditions, such as oxidative stress, calcium

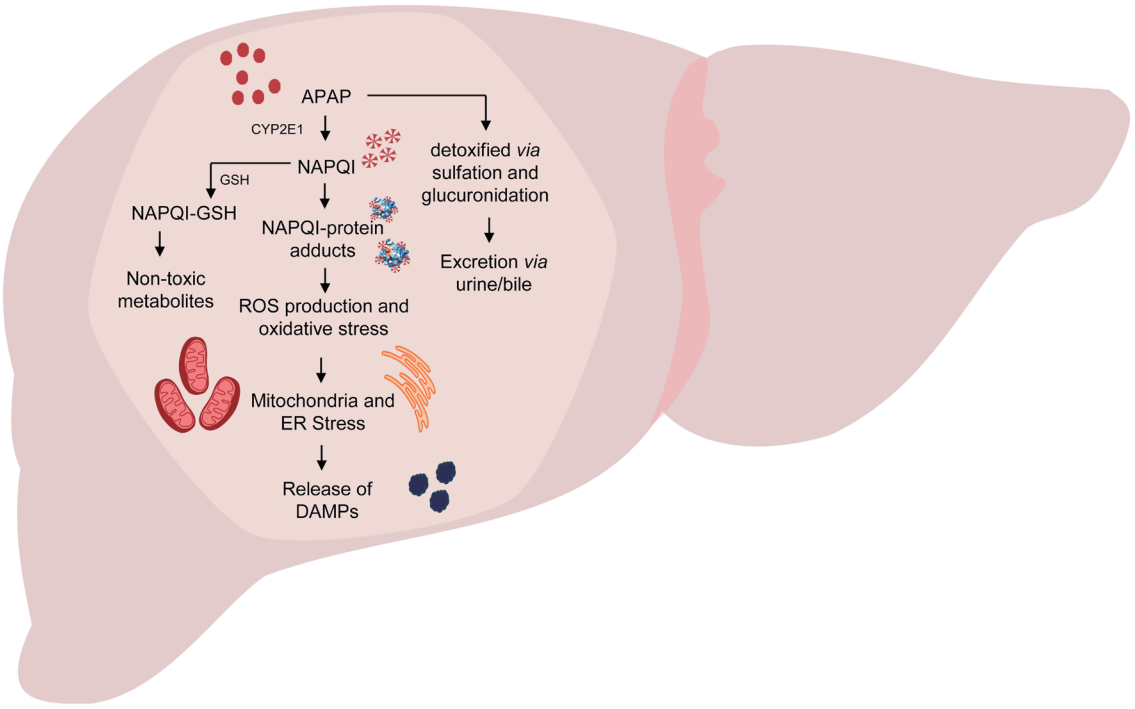

Fig. 1. Acetaminophen-induced hepatotoxicity: Under normal physiological conditions, APAP undergoes detoxification in the liver through sulfation and glucuronidation. A small fraction of APAP is converted by CYP2E1 into the highly reactive NAPQI, which is normally detoxified by GSH. However, under pathophysiological conditions, especially during APAP overdose, excessive NAPQI accumulates and binds to cellular proteins, also triggering oxidative stress, leading to hepatocellular damage and the release of damage-associated molecular patterns (DAMPs), contributing to further liver injury and inflammation. APAP, acetaminophen; CYP2E1, cytochrome P450 2E1; ER, endoplasmic reticulum; GSH, glutathione; NAPQI, N-acetyl-p-benzoquinone imine; ROS, reactive oxygen species.

overload, or energy depletion, leads to disruption of the mitochondrial membrane potential and the release of proapoptotic factors, ultimately culminating in cell death.[23] Some of the proteins released from mitochondria into the cytoplasm include apoptosis-inducing factor and endonuclease G, both of which have nuclear localization signals and thus translocate to the nucleus, where the endonuclease cleaves DNA, resulting in DNA fragmentation.[24,25] Similarly, apoptosis-inducing factor in the nucleus causes chromatin condensation and DNA fragmentation.[12,26] Furthermore, the formation of NAPQI adducts contributes to oxidative stress, mitochondrial dysfunction, disrupted Ca^{2+} homeostasis, necrotic cell death, centrilobular necrosis, and eventual liver failure (Figs. 3 and 4). Cellular swelling, karyolysis, karyorrhexis, vacuolization, release of cellular contents, endoplasmic reticulum stress, sterile inflammation, and autophagy are other mechanisms associated with APAP hepatotoxicity.[27]

Studies have identified p53, a tumor suppressor protein, as a protective factor against APAP-induced liver toxicity. In animal models of APAP overdose, inhibition of p53 has been shown to worsen liver injury, indicating that p53 plays a crucial role in mitigating the damage caused by APAP.[28] By activating p53, the liver initiates processes that promote cell cycle arrest, DNA repair, and apoptosis in damaged cells,[29] helping to limit the extent of liver injury and facilitating the removal of cells that have sustained significant damage. Inhibition of p53 disrupts these protective cellular mechanisms, allowing APAP-induced damage to progress unchecked, resulting in severe liver injury.[28] NAPQI has been shown to modify cysteine residues of the Kelch-like ECH-associated protein 1 (Keap1) protein, activating the Keap1-nuclear factor erythroid 2-related factor 2 (Nrf2) pathway.[30] Keap1 regulates the transcription factor Nrf2 by promoting its ubiquitination and degradation under normal conditions. During oxidative stress, Keap1 undergoes modifications that inhibit its interaction with Nrf2, allowing Nrf2 to accumulate and activate the expression of antioxidant and cytoprotective genes. This enhances cellular defense against oxidative damage and inflammation,[31] playing a crucial role in APAP-induced liver injury. When modified by NAPQI, Keap1 releases Nrf2, enabling its translocation into the nucleus, where it binds to antioxidant response elements in DNA and promotes transcription of genes encoding various antioxidant enzymes. These enzymes play a crucial role in combating NAPQI-induced oxidative stress by scavenging ROS and restoring cellular redox balance.[30,32] Consequently, activation of the Keap1-Nrf2 pathway serves as a defense mechanism to strengthen the antioxidant system and minimize the detrimental effects of oxidative stress in the liver caused by NAPQI.[30]

Acetaminophen-induced necroptosis and inflammation

The mechanism of cell death following APAP overdose has been the subject of extensive investigation for several decades. Early studies proposed necrosis as the predominant mode of cell death, based on hallmark features including karyorrhexis, karyolysis, plasma membrane rupture, and a robust inflammatory response.[33,34] However, evidence for apoptosis has only been partially validated, as it lacks both caspase activation and the formation of apoptotic bodies.[27,35–37] Moreover, despite the efficacy of potent caspase inhibitors, they failed to prevent APAP-induced cell death.[37]

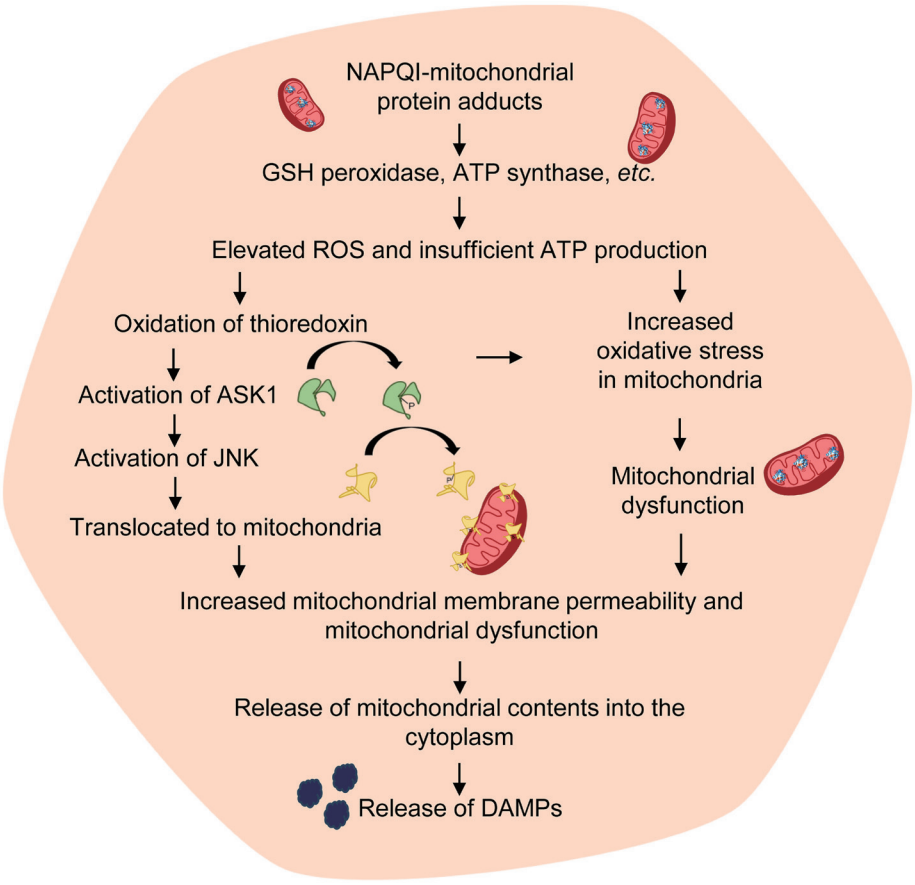

Fig. 2. Mitochondrial dysfunction: NAPQI binds to mitochondrial proteins, disrupting ATP synthesis and increasing ROS production. Oxidative stress activates the ASK1-JNK pathway, leading to JNK translocation to mitochondria, further exacerbating mitochondrial damage. This results in membrane instability and the release of DAMPs, triggering inflammation and hepatocyte injury. ASK1, apoptosis signal-regulating kinase 1; ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; GSH, glutathione; JNK, c-Jun N-terminal kinase; NAPQI, N-acetyl-p-benzoquinone imine; ROS, reactive oxygen species.

Necroptosis is a regulated form of necrotic cell death initiated by the binding of tumor necrosis factor (TNF)-α to its receptor TNFR1, forming a multiprotein signaling complex.[38] Under conditions where caspase-8 activity is inhibited or absent, receptor-interacting protein kinase 1 (RIPK1) interacts with RIPK3 to form the necrosome complex. Within this complex, RIPK3 is phosphorylated, which subsequently activates mixed lineage kinase domain-like protein (MLKL). Phosphorylated MLKL oligomerizes and translocates to the plasma membrane, disrupting membrane integrity and ultimately resulting in cell lysis and inflammation. RIPK3 levels have been shown to be elevated in APAP-overdosed mice[39]; however, MLKL knockout mice remained susceptible to APAP-induced liver injury, indicating that necroptosis is not the primary mode of cell death in APAP-induced toxicity.[34,40,41]

Ferroptosis, another form of regulated cell death characterized by iron-dependent accumulation of ROS, lipid peroxidation, and oxidative membrane damage, is increasingly recognized as a key contributor to APAP-induced liver injury.[42] Ferroptosis is initiated by the depletion of GSH, leading to the inactivation of GPx4, a key enzyme that protects against lipid peroxidation. In the absence of GPx4 activity, lipid peroxides accumulate within cellular membranes. Simultaneously, ferrous iron (Fe^{2+}) reacts with hydrogen peroxide (H_2O_2) via the Fenton reaction to generate highly reactive hydroxyl radicals (OH), which further propagate lipid peroxidation. This oxidative damage to membrane lipids compromises membrane integrity and ultimately results in cell death.[40] This mechanistic understanding highlights potential therapeutic avenues targeting ferroptosis for mitigating APAP-induced liver injury.[43] Ferroptosis inhibitors, such as ferrostatin-1 and UAMC-3203, were assessed for their ability to attenuate APAP hepatotoxicity. UAMC-3203 only partially attenuated liver injury by inhibiting the translocation of JNK protein and subsequently reducing mitochondrial damage and liver injury, while ferrostatin-1 demonstrated no protective effect. Hence, ferroptosis is likely not a major contributing factor in APAP-induced hepatotoxicity under physiological conditions.[44]

Pyroptosis has been reported as a significant mechanism of cell death in APAP-induced liver injury, involving activation of the NLRP3 inflammasome and subsequent caspase-1 activation. This cascade results in the cleavage of Gasdermin D, leading to the formation of membrane pores that may contribute to cell lysis and the release of the proinflammatory cytokines interleukin (IL)-1β and IL-18.[45–47] However, recent investigations have raised questions about the exclusive role of pyroptosis in APAP-induced hepatocyte loss.[48] Studies using Gasdermin D knockout models have demonstrated partial protection against hepatocyte loss, while pancaspase inhibitors failed to prevent cell death, although they reduced IL-1β production.[34,49,50] These findings suggest that APAP-induced cell

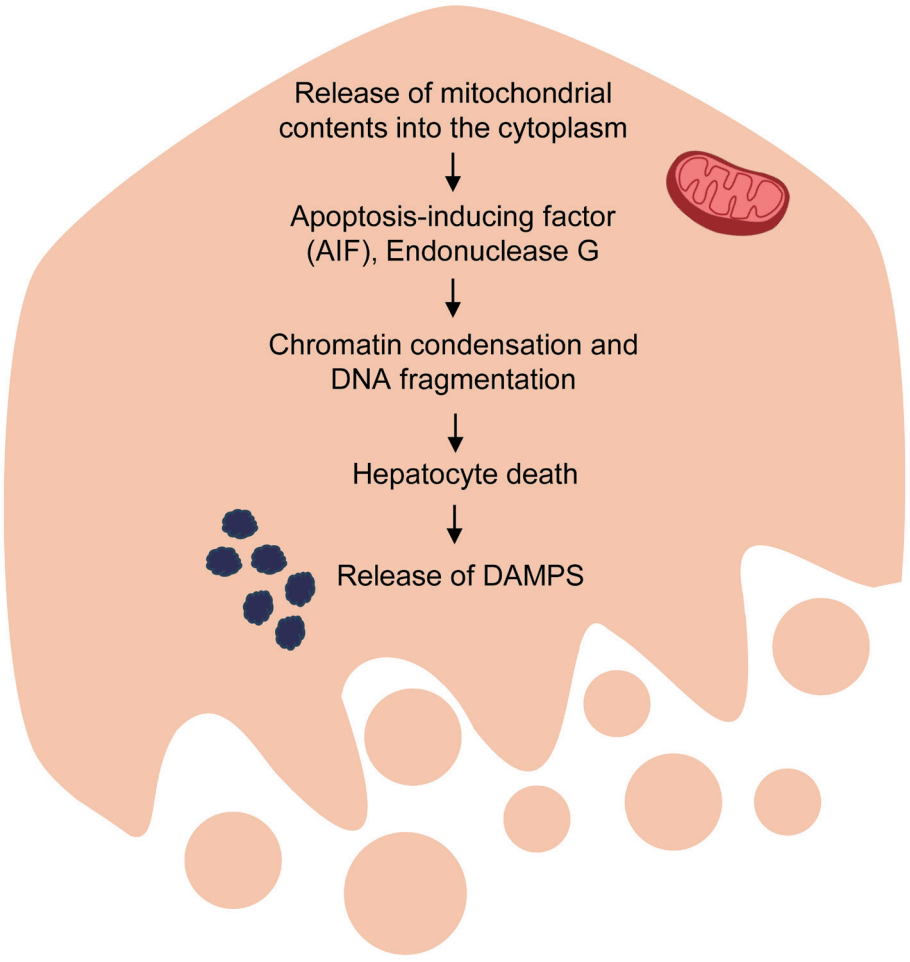

Fig. 3. Hepatocyte necrosis: apoptosis-inducing factor (AIF) and endonuclease G, along with other mitochondrial components, are released into the cytoplasm, causing chromatin condensation and DNA fragmentation. This ultimately leads to hepatocyte necrosis, triggering the release of DAMPs. DAMPs, damage-associated molecular patterns.

death may not strictly conform to conventional definitions of pyroptosis or other established modes of cell death, highlighting the complexity and distinct characteristics of liver injury induced by APAP.

During APAP overdose, damaged hepatocytes release damage-associated molecular patterns (DAMPs) such as high mobility group box-1 (HMGB1), ATP, and DNA fragments. These DAMPs activate the innate immune system, particularly Kupffer cells (liver-resident macrophages) and recruited monocytes, which release proinflammatory cytokines like TNF-α, IL-1β, and IL-6.[51] These cytokines amplify the inflammatory response. While the adaptive immune response is less immediate, there is evidence that it can be activated in the later stages of APAP-induced liver injury, contributing to sustained inflammation and liver damage.[52]

DAMPs, which include nuclear DNA fragments, ATP, HMGB1 protein, and mitochondrial DNA, are released into the bloodstream and recruit inflammatory cells to the site of liver injury. These include macrophages, neutrophils, natural killer cells, and natural killer T cells, which help clear necrotic cell debris and aid in liver cell recovery.[53,54] However, it has also been suggested that the enhanced release of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-10) and chemokines (monocyte chemoattractant protein-1, macrophage inflammatory protein-2, and IL-8) further promotes hepatocyte damage, thereby aggravating necrosis.[55] APAP-induced hepatocyte death triggers a neutrophilic inflammatory response, contributing to the worsening of existing injury.[56] Studies have also validated the role of neutrophils in the pathophysiology of APAP hepatotoxicity and the release of IL-1β, which is produced when caspase-1 cleaves pro-IL-1β.[56,57] The pathophysiological significance of this reaction to APAP is still controversial; whether it provides cellular protection against toxicity or worsens liver damage remains unclear. APAP-induced hepatotoxicity occurs in distinct phases, with inflammation acting as a promoter during the early stages. In later phases, inflammation facilitates the clearance of dead cells and cellular debris, thereby contributing to the stimulation of liver regeneration and repair.[58]

Inflammasome activation and its role in inflammatory responses

The inflammasome, a multiprotein complex, plays a central role in innate immunity and inflammatory responses by detecting cellular stress and danger signals, thereby coordinating the initiation of

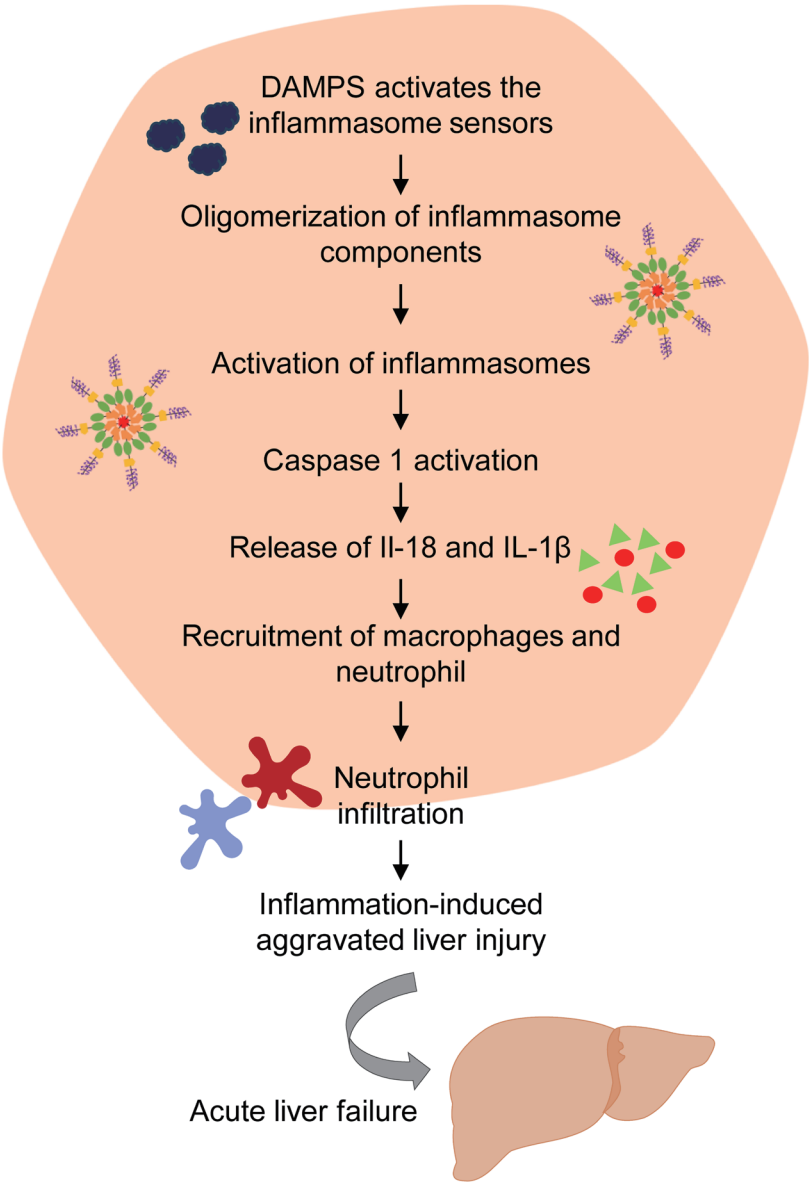

Fig. 4. Inflammasome-mediated liver injury: DAMPs released from stressed hepatocytes, damaged mitochondria, and necrotic liver cells activate inflammasome sensors, triggering the oligomerization and activation of inflammasome components. This process leads to caspase-1 activation, which facilitates the maturation and release of IL-18 and IL-1β. These proinflammatory cytokines recruit macrophages and neutrophils, intensifying inflammation and exacerbating liver injury, ultimately progressing to acute liver failure. IL, interleukin; DAMPs, damage-associated molecular patterns.

inflammatory pathways in response to diverse stimuli. The inflammasome complex comprises several key components, including a sensor, an adaptor, and an effector protein.[59] Inflammasomes are classified based on the sensor proteins that initiate their oligomerization. Some of the major types of inflammasomes characterized to date include NOD-, LRR-, and PYD domain-containing protein 3 (NLRP3), NOD-like receptor (NLR) family caspase activation and recruitment domain (CARD) domain-containing protein 4, absent in melanoma 2 (AIM2), NLRP1, NLRP6, and pyrin inflammasomes.[60] Each inflammasome type has a specific sensor protein and can respond to different stimuli, contributing to the regulation of inflammatory responses and immune homeostasis under various physiological and pathological conditions.[61]

The sensor protein recognizes specific danger signals or pathogen-associated molecular patterns and undergoes conformational changes upon activation.[60] The activated sensor protein recruits the adaptor protein, which acts as a scaffold for assembling the inflammasome complex. Several families of sensor proteins, including NLRs, AIM2-like receptors, and pyrin, are involved in forming inflammasome complexes. Each sensor protein recognizes specific ligands and undergoes conformational changes upon ligand binding to initiate inflammasome assembly.[61] The most commonly studied adaptor protein is apoptosis-associated speck-like protein containing a CARD (ASC), which contains a CARD that interacts with the CARD domain of pro-caspase-1.[62] The effector protein is typically pro-caspase-1, an inactive precur-

sor of caspase-1. Pro-caspase-1 is recruited to the complex upon inflammasome assembly and undergoes autoproteolytic cleavage to generate active caspase-1.[60] Their primary function is to detect molecules known as DAMPs and pathogen-associated molecular patterns.[63] By recognizing these signals, inflammasomes play a crucial role in initiating and regulating the inflammatory response in the body, particularly in response to cellular damage or microbial threats.[61] Finally, the effector protein, typically a caspase enzyme, is recruited to the complex and activated, initiating downstream signaling events that lead to inflammation and cell death. One well-characterized inflammasome complex is the NLR family, which includes the prototypical NLRP3 inflammasome. NLRP3 senses a wide array of endogenous and exogenous danger signals, including microbial products, environmental toxins, and host-derived DAMPs, making it a central player in inflammatory responses.[64] Inflammasome complex activation is a tightly regulated process involving multiple steps. First, the sensor protein within the inflammasome complex recognizes specific danger signals or stressors, triggering its activation. This induces a conformational change in the sensor protein, allowing it to recruit the adaptor protein and initiate the assembly of the inflammasome complex. Once assembled, the inflammasome complex serves as a platform for activating caspase enzymes, particularly caspase-1.[65] Activated caspase-1 cleaves the proinflammatory cytokines pro-IL-1β and pro-IL-18 into their active forms, promoting inflammation and immune responses.[65,66] Additionally, caspase-1 activation leads to programmed cell death known as pyroptosis, characterized by cell swelling, membrane rupture, and the release of proinflammatory intracellular contents.[63]

Role of inflammasome activation in APAP-induced liver injury

Inflammasome activation has been implicated in the pathogenesis of various hepatic and systemic inflammatory diseases, including nonalcoholic fatty liver disease,[67–69] alcoholic liver disease,[70,71] viral hepatitis,[72] and sepsis-induced liver injury.[73] During pathogenesis, inflammasomes are activated in response to damage-associated molecular patterns and pathogen-associated molecular patterns, releasing proinflammatory cytokines such as IL-1β and IL-18.[59] Furthermore, chronic inflammasome activation has been linked to metabolic disorders such as obesity and type 2 diabetes, contributing to systemic inflammation and hepatic dysfunction.[74] The pathophysiology of APAP-induced liver injury involves a complex interplay of cellular events, with inflammasome activation emerging as a critical component of the inflammatory response.[8] APAP overdose initiates a cascade of events within the liver. Under normal conditions, NAPQI is detoxified by conjugation with GSH. However, during APAP overdose, the excessive production of NAPQI overwhelms the cellular antioxidant capacity, leading to the accumulation of NAPQI-protein adducts.[5] This process induces oxidative stress and mitochondrial dysfunction, resulting in hepatocyte necrosis and the release of cellular contents, including DAMPs. During the early stages of APAP-induced liver injury, stressed and damaged hepatocytes release various endogenous DAMPs, such as HMGB1,[75] nuclear DNA fragments,[76] uric acid,[77] mitochondrial DNA,[76] ATP, and heat shock protein-70, an inducible stress response protein that is translocated to the cell surface and released during cellular stress or necrotic death.[78] These DAMPs play a major role in promoting neutrophil infiltration by activating antigen-presenting cells, including Kupffer cells and dendritic cells, which amplifies the immune response and the progression of liver injury.[78] These DAMPs act as danger signals that activate innate immune receptors, including pattern recognition receptors. Among these receptors, NLRP3 plays a central role in sensing cellular stress and danger signals.[79] NLRP3 activation requires two distinct signals: priming and activation. The priming signal involves the upregulation of NLRP3 expression and the synthesis of proinflammatory cytokines such as TNF-α and IL-1β, typically mediated by nuclear factor kappa B activation in response to inflammatory stimuli.[80] Several DAMPs, including ATP, mitochondrial DNA, and ROS, serve as activation signals (Fig. 3). The NLRP3 inflammasome is a critical component of the innate immune system responsible for detecting cellular stress and damage signals. The upstream and downstream regulators of NLRP3 in APAP-induced liver injury are summarized in Tables 1 and 2.[48,81–124]

Upon sensing the activation signal, NLRP3 recruits the adaptor protein ASC and pro-caspase-1, leading to inflammasome complex assembly. This multimeric complex facilitates the autocatalytic activation of caspase-1, an inflammatory caspase, which cleaves pro-IL-1β and pro-IL-18 into their mature, biologically active forms, IL-1β and IL-18. These cytokines are potent mediators of inflammation and play crucial roles in orchestrating innate and adaptive immune responses.[79] The release of mature IL-1β and IL-18 amplifies the inflammatory response in the liver, leading to the recruitment and activation of immune cells, including neutrophils, monocytes, and macrophages. These cells produce additional proinflammatory mediators, such as TNF-α, IL-6, and ROS, further exacerbating liver inflammation and hepatocyte injury. The sustained inflammatory cascade ultimately contributes to hepatocyte death, liver dysfunction, and progression to acute liver failure in severe cases of APAP overdose (Fig. 4).

Inflammasomes are reported to be involved primarily in sterile inflammation, promoting the removal of necrotic cellular debris, hepatocyte proliferation, and liver tissue regeneration.[54] A recent study demonstrated that cross-talk between necroptosis and the NLRP3 inflammasome promotes APAP-induced liver injury.[125] Targeting inflammasomes in APAP-induced liver injury has emerged as a potential therapeutic strategy to mitigate inflammation, tissue damage, and liver injury. NLRP3 activation in the liver results in shortened survival, hepatocyte pyroptosis, severe liver inflammation, and neutrophil infiltration.[46,125,126] NLRP3$^{-/-}$, Casp1$^{-/-}$, and ASC$^{-/-}$ mice are less susceptible to APAP-induced liver injury and show reduced mortality.[57] Genetic ablation of NLRP3 significantly reduces the release of cleaved IL-1β and IL-18, thereby alleviating APAP-induced liver injury.[57] Similarly, inhibiting NLRP3 by suppressing Toll-like receptors (TLRs) 4 and 9 signaling pathways also results in hepatoprotection against APAP-induced liver injury.[57,127,128] Suppressing NLRP3 inflammasome activation, ASC speck formation, caspase-1 activation, and IL-1β maturation alleviates acute liver injury caused by APAP poisoning.[129–131] The administration of pharmacological inhibitors targeting inflammasome components, such as NLRP3 or caspase-1 inhibitors, mitigates APAP-induced liver injury in preclinical models, highlighting the pivotal role of inflammasomes in modulating hepatic inflammation and damage.[132]

Several phytocompounds, such as Sapidolide A,[47] allicin,[133] and berberine,[134] have been shown to inhibit inflammasome activation and alleviate APAP-induced hepatotoxicity. Additionally, oridonin, identified as a covalent inhibitor of inflammasomes, has demonstrated significant efficacy in mitigating damage resulting from APAP overdose.[135] AIM2 deficiency, another sensor involved in inflammasome activation, enhances APAP-induced inflamma-

Table 1. Overview of the upstream regulators of NLRP3 in APAP-induced liver injury

Upstream regulators	Mechanism of action
Reactive oxygen species (ROS)	
Mitochondrial ROS	APAP metabolism generates ROS from mitochondria, directly activating NLRP3[82,83]
CYP2E1 activity	Cytochrome P450 2E1 metabolizes APAP into reactive intermediates, increasing oxidative stress and ROS[84,85]
Mitochondrial dysfunction	
Mitochondrial damage	APAP overdose induces mitochondrial damage and mtDNA release, acting as DAMPs to activate NLRP3[86–88]
mPTP opening	Mitochondrial permeability transition pore opening releases proapoptotic factors, promoting dysfunction and NLRP3 activation[89–91]
Ion flux	
Potassium efflux	APAP-induced cellular damage causes potassium efflux, triggering NLRP3 activation[81,91]
Calcium signaling	Disruptions in calcium homeostasis further promote NLRP3 activation[92,93]
Lysosomal destabilization	
Lysosomal damage	Oxidative stress from APAP causes lysosomal membrane permeabilization, releasing cathepsins to activate NLRP3[94,95]
High-mobility group box 1 (HMGB1)	
HMGB1 release	Necrotic hepatocytes release HMGB1, a DAMP that activates TLRs and subsequently NLRP3[96–98]
ATP release	
Extracellular ATP	Damaged hepatocytes release ATP, activating P2X7 receptor, leading to potassium efflux and NLRP3 activation[99,100]
Cytokines and chemokines	
IL-1β and TNF-α	IL-1β and TNF-α amplify the inflammatory response and upregulate NLRP3 *via* NF-κB signaling[101]
NADPH oxidase	
NADPH oxidase activity	Contributes to ROS production, promoting NLRP3 activation[102,103]
MicroRNAs	
miR-223	Downregulation of miR-223, which suppresses typically NLRP3, enhances inflammasome activation[104,105]
Transcription factors	
NF-κB	Upregulates NLRP3 and pro-IL-1β expression in response to inflammatory stimuli[101,106,107]
STAT3	Modulates the expression of inflammasome components, influencing NLRP3 activity[108]

APAP, acetaminophen; CYP2E1, cytochrome P450 2E1; DAMPs, damage-associated molecular patterns; HMGB1, high mobility group box-1; IL, interleukin; mPTP, mitochondrial permeability transition pore; mtDNA, mitochondrial DNA; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa B; NLRP3, NOD-, LRR-, and pyrin domain-containing 3; P2X7, purinergic receptor P2X 7; STAT3, signal transducer and activator of transcription 3; TLRs, toll like receptors; TNF-α, tumor necrosis factor-alpha.

tion and acute liver injury in aged mice by promoting oxidative stress and phosphorylation of JNK.[136] Experimental models provide compelling evidence connecting inflammasome activation to APAP-induced liver injury. However, the role of inflammasomes in this injury remains unclear, as conflicting findings suggest inflammasomes may exacerbate liver injury. Endogenous IL-1β has been reported to be insufficient for activating and recruiting neutrophils, and even high pharmacological doses of IL-1β fail to exacerbate APAP-induced liver injury, indicating that IL-1 signaling is not a critical contributor to APAP-induced hepatotoxicity.[49] Moreover, administration of high doses of IL-1β fails to exacerbate APAP-induced liver injury, further suggesting a limited role of IL-1 signaling in the pathogenesis of APAP hepatotoxicity.[49,137] Consistently, IL-1 receptor-deficient (IL-1R$^{-/-}$) mice show no significant protection following APAP overdose.[49] Similarly, genetic ablation of NLRP3 provides no evidence of hepatoprotection.[138] Inhibition of IL-1β maturation using pan-caspase inhibitors reduces circulating IL-1β levels without attenuating neutrophil infiltration, inflammation, or liver injury.[48]

Mice deficient in ASC, NLRP3, or caspase-1 fail to demonstrate significant protection against APAP-induced liver injury. The extent of hepatic damage, along with levels of proinflammatory cytokines, chemokines, and neutrophil infiltration, remains comparable to that observed in wild-type controls.[137] Although recent studies support a role for NLRP3 inflammasomes in APAP-induced liver injury, the discrepancies and complexity of available data underscore the need for further research. Future investigations should focus on understanding the role of NLRP3 at different phases of APAP-induced liver injury, its activation in various cell types, including hepatocytes and Kupffer cells, and validating its involvement in liver damage in multiple animal models through comparative analyses. Further research is necessary to assess the therapeutic potential of NLRP3 inhibitors, particularly when combined with other medications.

Table 2. Overview of the downstream regulators of NLRP3 in APAP-induced liver injury

Downstream regulators	Mechanism of action
Caspase-1 activation	
Caspase-1	Activated by NLRP3 inflammasome, leading to the cleavage and activation of proinflammatory cytokines IL-1β and IL-18[109,110]
Proinflammatory cytokines	
IL-1β	Cleaved and activated by caspase-1, leading to the recruitment of inflammatory cells and amplification of the inflammatory response[111,112]
IL-18	Also cleaved and activated by caspase-1, contributing to the inflammatory response and immune cell recruitment[112]
Pyroptosis	
Gasdermin D	Cleaved by activated caspase-1, forming pores in the cell membrane that lead to pyroptosis, a form of inflammatory cell death[113–115]
Recruitment of immune cells	
Neutrophils and macrophages	IL-1β and IL-18 promote the recruitment and activation of neutrophils and macrophages, exacerbating liver inflammation and damage[116]
Inflammatory mediators	
HMGB1 release	Further amplifies inflammation by acting as a danger-associated molecular pattern[117,118]
Tissue damage and fibrosis	
Hepatocyte death	Pyroptosis and inflammatory cell infiltration lead to hepatocyte death and liver tissue damage[48,119]
Fibrogenesis	Chronic inflammation can stimulate hepatic stellate cells, leading to fibrosis[120,121]
Systemic inflammatory response	
Acute phase response	Elevated levels of proinflammatory cytokines can trigger a systemic inflammatory response, affecting multiple organs[122]
Regulation of adaptive immunity	
Dendritic cell activation	IL-1β and IL-18 can enhance dendritic cell maturation and antigen presentation, linking innate and adaptive immunity[123,124]

APAP, acetaminophen; HMGB1, high mobility group box-1; IL, interleukin; NLRP3, NOD-, LRR-, and pyrin domain-containing 3.

Therapeutic implications

Therapeutic strategies for APAP-induced liver injury involve mitigating inflammation, preventing hepatocyte death, and promoting liver regeneration. Several approaches have been explored, including targeting inflammasome activation, inhibiting caspase-1, employing anti-inflammatory agents, and modulating the gut microbiota and bile acid metabolism. Inhibition of the NLRP3 inflammasome represents a promising therapeutic strategy for attenuating inflammation and reducing tissue damage in APAP-induced liver injury. Various small-molecule inhibitors targeting different stages of NLRP3 activation have been developed and investigated preclinically; these are listed in Table 3.[47,131,133,139–149] These inhibitors may target upstream signaling events, such as potassium efflux,[81] mitochondrial dysfunction,[150] or lysosomal destabilization,[151,152] all critical for NLRP3 activation. Small-molecule inhibitors such as MCC950 have shown efficacy in inhibiting NLRP3 activation and ameliorating liver injury in experimental models of APAP overdose.[140] Scopoleitin mitigates APAP-induced hepatotoxicity through suppression of NLRP3 gene expression and concurrent inhibition of the Nrf2/HMGB1/TLR4/NF-κB signaling pathway, resulting in reduced levels of proinflammatory factors.[141] Piperine exhibits a prophylactic effect against APAP-induced liver injury by modulating inflammasome activation, reducing oxidative stress and cellular necrosis, and ultimately limiting activation of pro-IL-1β.[142] Aloperine inhibits HMGB1/TLR4/NF-κB and NLRP3 inflammasome activation, ameliorating oxidative stress and inflammation, thereby mitigating APAP-induced liver injury.[143] Salidroside downregulates the levels of NLRP3, ASC, caspase-1, and IL-1β and significantly protects the liver from APAP overdose in C57BL/6 mice.[144] Similarly, Pien Tze Huang, a traditional Chinese medicine, also exhibits protective effects against APAP-induced liver injury by significantly reducing levels of NLRP3 inflammasomes and proinflammatory cytokines.[145] Rosmarinic acid demonstrates a hepatoprotective role through inhibition of Nrf2-mediated downregulation of the NIMA (never in mitosis gene a)-related kinase 7-NLRP3 pathway.[146] Sapidolide A, extracted from a folk medicine used in China to treat inflammatory diseases, inhibits expression and activation of the NLRP3 inflammasome when used as a pretreatment in a mouse model of APAP-induced liver injury.[47] Emodin exerts anti-inflammatory effects by inhibiting the NLRP3 pathway, thereby preventing hepatotoxicity induced by APAP.[147] Peroxiredoxin 3 modulates NLRP3-induced pyroptosis by regulating mitochondrial oxidative stress and inflammation, mitigating APAP-induced liver injury.[148] Kaempferol inhibits HMGB1 and TLR4 and downregulates NLRP3 expression, reducing proinflammatory cytokine levels and preventing APAP hepatotoxicity.[131] Similarly, sinomenine exhibits hepatoprotective effects through suppression of NLRP3 inflamma-

Table 3. NLRP3 inhibitors and their mechanism of action against acetaminophen-induced liver injury

Compounds	Mechanism of action
Scopoleitin	Ameliorates the AILI through HMGB1/TLR4/NLRP3 inflammasome pathway[141]
Piperine	Protects the liver from AILI by modulating the inflammasome pathway[142]
Aloperine	Reduced oxidative stress and inflammation through the inhibition of HMGB1/TLR4/NF-κB and NLRp3 inflammasome pathway[143]
Salidroside	Downregulates the NF-κB/ NLRp3 inflammasome axis, thereby protecting the liver from acetaminophen-induced hepatotoxicity[144]
Pien Tze Huang	Inhibits NLRP3 inflammasome and protects the liver from damage[145]
Rosmarinic acid	Suppresses the APAP-induced NEK7-NLRP3 activation and regulates the Nrf2-mediated inhibition of ROS production[146]
Sapidolide A	Regulates the NLRP3 activation in macrophages and exerts anti-inflammatory effects[47]
Emodin	Upregulates Nrf2-mediated antioxidative pathway and inhibits NLRP3 activation[147]
Peroxiredoxin 3	Targets mitochondrial oxidative stress and inhibits NLRP3 activation[148]
Kaempferol	Inhibits HMGB1, TLR4, and NF-κB activation and regulates the NLRP3 expression[131,149]
Sinomenine	Inhibits the activation of NLRP3 inflammasomes, reduces the inflammatory cytokine levels
Allicin	Inhibits the NLRp3 signaling reduced pro-caspase-1, cleavage and IL-1β release[133]
MCC950	Blocks the NLRP3/GSDMD signaling and ameliorates the liver injury and hepatocyte loss[140]

AILI, acetaminophen induced liver injury; GSDMD, Gasdermin D; HMGB1, high mobility group box-1; NF-κB, nuclear factor kappa B; NLRP3, NOD-, LRR-, and pyrin domain-containing 3; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; TLR4, Toll-like receptors 4.

some activation, reduction of oxidative stress, and inhibition of the HMGB1/TLR4/NF-κB pathway.[153] Allicin also demonstrates hepatoprotective effects against APAP-induced liver injury by attenuating oxidative stress and inhibiting the NLRP3 inflammasome pathway, effectively preventing the release of proinflammatory cytokines.[133] Caspase-1 is a key effector molecule downstream of inflammasome activation and plays a central role in processing and releasing proinflammatory cytokines, such as IL-1β and IL-18. Pharmacological inhibitors targeting caspase-1 activity have been investigated for their potential to dampen the inflammatory response and reduce tissue damage in APAP-induced liver injury. In preclinical studies, compounds such as VX-765 and pralnacasan have demonstrated efficacy in inhibiting caspase-1 activation and protecting against inflammatory diseases.[154] However, further research is needed to evaluate their safety and efficacy in clinical settings. Anti-inflammatory agents, such as corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), have been explored for their potential to attenuate inflammation and mitigate liver injury in APAP overdose.[155,156] While corticosteroids have shown limited efficacy and potential adverse effects in clinical trials, NSAIDs, particularly those with selective cyclooxygenase-2 inhibition, have demonstrated some protective effects in experimental models. However, the use of NSAIDs in APAP overdose remains controversial due to concerns about exacerbating hepatotoxicity and gastrointestinal bleeding. Similarly, cyclosporin A, a well-characterized immunosuppressive agent, has demonstrated protective effects against APAP-induced hepatotoxicity when administered in combination with NAC in murine models.[157] While corticosteroids and other immunosuppressive agents exert broad anti-inflammatory effects, direct inhibition of NLRP3 inflammasome activation represents a more specific therapeutic strategy, potentially allowing for more effective suppression of inflammation with reduced off-target effects.

Emerging evidence suggests that the gut-liver axis plays a critical role in the pathogenesis of APAP-induced liver injury, and modulation of the gut microbiota and bile acid metabolism represents a novel therapeutic approach. Preclinical studies have shown that antibiotics, probiotics, and fecal microbiota transplantation can alter the gut microbiota composition and reduce liver injury in APAP overdose by modulating inflammatory responses and enhancing hepatocyte regeneration.[155] Additionally, targeting bile acid metabolism, such as through bile acid sequestrants and farnesoid X receptor agonists, has shown promise in preclinical models by reducing bile acid accumulation and hepatotoxicity. Targeting inflammasome activation, inhibiting caspase-1, employing anti-inflammatory agents, and modulating the gut microbiota and bile acid metabolism represent promising avenues for developing novel treatments for this common and potentially life-threatening condition. Further research is needed to validate the efficacy and safety of these approaches in clinical settings and to identify optimal therapeutic regimens for patients with APAP overdose.

This review is inherently limited by its predominant focus on preclinical studies conducted in animal models or *in vitro* systems. While such models provide significant mechanistic insights into APAP-induced hepatotoxicity, including pathways related to oxidative stress, mitochondrial dysfunction, and inflammasome activation, they may not fully recapitulate the complexity of human physiology. Inter-species differences in drug metabolism, immune responses, and liver regeneration capacity can affect the translatability of findings, thereby restricting the extrapolation of preclinical data to clinical settings. Furthermore, the absence of strong data from clinical trials on novel therapeutic targets, such as inflammasome inhibitors or microbiome-modulating agents, limits the strength of conclusions regarding their efficacy and safety in humans. Although several drug molecules have shown hepatoprotective effects in experimental conditions, their clinical use remains inconclusive until validated in well-designed clinical trials. Another notable limitation is the scope of the populations discussed. This review does not comprehensively address APAP toxicity in specific vulnerable populations, such as neonates, pedi-

atric patients, pregnant women, or the elderly, who may exhibit distinct pharmacokinetics, pharmacodynamics, and susceptibility to liver injury. These populations may require unique therapeutic approaches and risk stratification methods that were beyond the scope of the current review. Future studies should prioritize translational research efforts that bridge preclinical discoveries with clinical application, as well as investigate APAP-induced hepatotoxicity across diverse demographic and physiological subgroups.

Conclusions

Acetaminophen-induced liver injury remains a significant global public health concern due to its potential to cause acute liver failure and mortality in severe cases. The hepatotoxic effects of APAP are primarily mediated by its bioactivation to the reactive metabolite NAPQI via CYP2E1. Excessive accumulation of NAPQI depletes hepatic GSH stores, leading to oxidative stress, mitochondrial dysfunction, and covalent modification of cellular macromolecules. Multiple regulated and unregulated cell death pathways contribute to APAP-induced hepatotoxicity, including necrosis, apoptosis, necroptosis, and pyroptosis. Among these, sterile inflammation plays a pivotal role in amplifying liver injury. Inflammasome complexes, particularly NLRP3, have emerged as key regulators of this inflammatory response. Activation of the NLRP3 inflammasome leads to the cleavage of procaspase-1 into its active form, which in turn processes proinflammatory cytokines such as IL-1β and IL-18 and drives pyroptotic cell death *via* Gasdermin D activation. Despite the availability of NAC as the primary therapeutic agent for APAP overdose, treatment options remain limited, particularly when administered beyond the early therapeutic window. Consequently, current research has focused on identifying novel therapeutic strategies targeting the inflammatory and cell death pathways involved in APAP-induced hepatotoxicity. These include direct inhibition of inflammasome components, caspase-1 blockade, modulation of immune responses using anti-inflammatory agents, and targeted manipulation of gut microbiota and bile acid metabolism, which are increasingly recognized for their roles in liver homeostasis and injury response. Pharmacological inhibitors such as NLRP3 antagonists and caspase-1 inhibitors show promise in preclinical models but require further clinical validation. Emerging strategies targeting the gut-liver axis, such as microbiome modulation and bile acid receptor agonists, also offer new therapeutic avenues for APAP-induced liver injury. However, a deeper understanding of the molecular mechanisms leading to APAP-induced liver injury is crucial for developing effective, timely, and targeted therapies to reduce the global burden of APAP toxicity.

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Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Concept and design, reference collection, and writing of the manuscript (NS, LC). Both authors have approved the final version and publication of the manuscript.

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