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# Damage-Associated Molecular Patterns: Their Impact on the Liver and Beyond During Acetaminophen Overdose

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Acetaminophen (APAP) is one of the most commonly used drugs for treating pain and fever. Although it is safe at therapeutic dosage levels, overdose of APAP causes severe liver injury (APAP-induced liver injury; AILI), with the potential to progress to liver failure. Up to 40% of patients who suffer from liver failure will die (or undergo liver transplant).[1] Data combined from 22 specialty medical centers in the United States revealed that AILI accounts for approximately half of acute liver failure cases and results in more than 56,000 emergency room visits, 2,600 hospitalizations, and an estimated 458 deaths each year.[1]

APAP hepatotoxicity is initiated by the generation of a chemically reactive metabolite (*N*-acetyl-*p*-benzoquinone imine; NAPQI). NAPQI depletes liver glutathione and covalently binds to cellular proteins, thereby causing mitochondrial dysfunction.[2-6] Studies using rodent models in the last four decades have demonstrated that mitochondrial disruption is the key underlying mechanism of AILI (Fig. 1FIG1)). Covalent binding to mitrochondrial proteins by NAPQI causes oxidative stress and mitochondrial membrane permeability transition pore opening, which triggers the collapse of membrane potential, cessation of adenosine triphosphate (ATP) production, and the release of apoptosis-inducing factor and endonuclease G.[7-11] Together, the mitochondrial dysfunction, energy crisis, and nuclear DNA damage result in hepatocyte necrosis.

In recent years, there has been a growing interest to investigate whether downstream events of early hepatocyte necrosis contribute to the aggravation and progression of AILI. Necrotic cells release a number of damage-associated molecular pattern (DAMP) molecules, such as high-mobility group box-1, heat-shock proteins, hyaluronan, fibronectin, cardiolipin, and DNA fragments. Upon activation by DAMP molecules, innate immune cells infiltrate the damaged area and release cytokines and cheomokines, thereby causing tissue sterile inflammation.[12-22] The soluble products of innate immune cells can exacerbate tissue damage, as well as promote wound healing (Fig. 1). Perhaps as a result of this dichotomy, the overall contribution of innate immune cells, such as neutrophils, to AILI remains unclear.

In this issue of HEPATOLOGY, Marques et al.[23] report on a study demonstrating that DAMP molecules released from necrotic hepatocytes recruit and activate neutrophils in the liver, which, in turn, amplify AILI. Three experimental approaches were employed to elucidate the pathological role of neutrophils in AILI. Consistent with published reports,[24,25] the present study shows that neutrophil depletion by an anti-Gr-1 antibody significantly attenuates AILI. Furthermore, the combined use of a CXC chemokine receptor 2 antagonist and a formyl peptide receptor 1 (FPR1) antagonist also blocks hepatic recruitment of neutrophils and mitigates AILI. This approach is based on the investigators' previous finding that an intravascular gradient of chemokines and mitochondria-derived formyl peptides collaboratively guide neutrophils to sites of liver necrosis.[26] These two separate *in vivo* studies demonstrate that liver injury initiated by APAP challenge is amplified by infiltrating neutrophils. The investigators further examined the cytotoxic potential of neutrophils against hepatocytes. Neutrophils isolated from healthy individuals were cocultured with APAP-treated HepG2 cells. Data show that the cytotoxicity of HepG2 cells is enhanced by neutrophils in a cell-contact–dependent manner, and that necrotic HepG2 cells significantly increase reactive oxygen species production by netrophils. Collectively, these findings provide evidence to support a pathological role of neutrophils during AILI.

Traumatic injury is known to cause "septic-like" systemic inflammatory response in the absence of infection.[27] The underlying mechanism is recently

*Abbreviations: AILI, APAP-induced liver injury; APAP, acetaminophen; ATP, adenosine triphosphate; DAMP, damage-associated molecular pattern; FPR1, formyl peptide receptor 1; IL, interleukin; mtDNA, mitrochondrial DNA; NAPQI,* N*-acetyl*-p*-benzoquinone imine; TLR-9, Toll-like receptor 9.*

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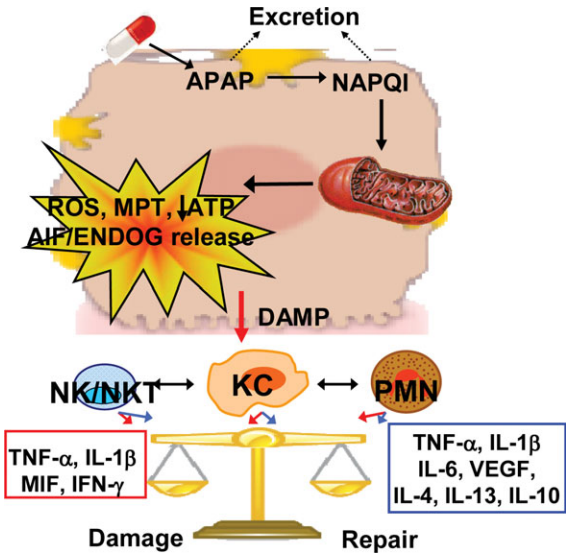


Fig. 1. Mechanism of AILI. Metabolic activation of APAP generates NAPQI. Glutathione depletion and protein binding by NAPQI cause mitochondrial oxidative stress, mitochondrial permeability transition (MPT), ATP depletion, and release of apoptosis-inducing factor (AIF) and endonuclease G (ENDOG), all of which culminate in hepatocyte necrosis. DAMPs are released and trigger the activation of innate immune cells, such as neutrophils (PMN), Kupffer cells (KC), and natural killer (NK) and NK T cells. Some soluble factors produced by activated innate immune cells can cause further tissue damage, such as tumor necrosis factor (TNF)-$\alpha$, IL-1$\beta$, interferon (IFN)-$\gamma$, and macrophage migration inhibitory factor (MIF). However, the same factors (TNF-$\alpha$ and IL-1$\beta$) and other mediators, including IL-6, IL-4, IL-13, IL-10, and vascular endothelial growth factor (VEGF), are important in promoting liver repair.

elucidated by the detection of mitrochondrial DNA (mtDNA) and formyl peptides released in the serum of trauma patients.[28] Circulating mitochondrial DAMPs activate neutrophils through Toll-like receptor 9 (TLR-9) and FPR1, respectively, thereby eliciting neutrophil-mediated organ injury.[28] Because APAP causes mitochrondria damage, it is likely that mitochondrial contents are released into the circulation. Evidence supporting this hypothesis is provided by a recent study detecting circulating mitochondrial biomarkers, including mtDNA and glutamate dehydrogenase, in the serum of patients with AILI.[29] Similarly, the present study shows a significant increase in serum mtDNA levels in acute liver failure patients, compared to healthy volunteers. The elevation of circulating mtDNA is also observed in APAP-treated mice. The effect of mtDNA release on AILI is revealed by a significant decrease of liver injury in TLR-9$^{-/-}$ mice, compared to wild-type mice. This finding is consistent with a published report of protection against AILI by TLR-9 antagonists and in TLR-9$^{-/-}$ mice.[30]

APAP-induced liver failure is accompanied by other tissue complications, such as encephalopathy, coagulopathy, renal failure, metabolic derangements, cardiovascular compromises, and severe lung injury.[31,32] Toxic effects on remote tissues may be the result of the original insult of APAP, but, in many cases, are consequences of severe liver injury. A novel finding of the present study is the observation of marked lung injury in mice treated with APAP. Data suggest that lung injury is the result of the systemic release of mitochondrial DAMPs, because blockade of FPR1 or deletion of TLR-9 significantly reduced lung injury. From their results, the investigators summarize a mechanistic model for APAP-induced liver damage and lung inflammation. APAP-initiated hepatocyte necrosis causes the release of mitochondrial DAMPs and chemokines, which lead to hepatic recruitment of neutrophils that amplify liver damage. The release of mitochondrial DAMPs also triggers systemic inflammation and causes organ injury at remote sites.

Many studies clearly support that neutrophils are recruited into the liver after cellular damage initiated by APAP challenge. However, the key unresolved question is whether or not the infiltrated neutrophils are activated and aggravate AILI. Data from some studies, including the present one, provide evidence for a pathological role of neutrophils in AILI. However, other studies have demonstrated that (1) neutrophils recruited into the liver are not activated,[33] (2) blocking neutrophil recruitment does not affect AILI,[33,34] and (3) even activating neutrophils by endotoxin or interleukin (IL)-1$\beta$ does not worsen AILI.[33,35] Aside from the dichotomy of tissue-damaging and -repair functions of neutrophils, these discrepancies can be, at least in part, explained by different experimental protocols employed by various research groups. For example, two critical experimental conditions that can significantly affect the severity and kinetics of AILI include the mouse strains, as well as the dose and route of administration of APAP. Therefore, direct comparisons can only be made when the experimental approaches are unified.

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