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Review Article on Acetaminophen-Induced Dose-Dependent Hepatotoxicity and Mechanism Involved in *In Vivo* and *In Vitro* Studies

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ABSTRACT

Drug-induced liver damage is an uncommon but important clinical issue. At therapeutic levels, a variety of medications can result in serious liver damage and abrupt liver failure in a very small proportion of people (1:10,000). This idiosyncratic medication-induced liver damage, which is presently unpredictable in preclinical safety investigations, appears to depend on individual vulnerability and the incapacity to adapt to the cellular stress imposed by a specific treatment. Drugs with dose-dependent hepatotoxicity are often discovered during preclinical trials and are not commercially available, in stark contrast to idiosyncratic drug-induced liver disease. Acetaminophen is one prominent exception; while it is safe at therapeutic levels, overdosing on it can result in severe liver damage and abrupt liver failure. Based on early knowledge of APAP toxicity in mice, N-acetylcysteine was quickly developed as an antidote to APAP overdose. N-acetylcysteine is effective in treating APAP overdose patients who come right away, but there is still a need to create intervention plans for patients who present much later.

INTRODUCTION:

One of the medications with the greatest research has been acetaminophen, also known as N-acetyl-para-aminophenol (APAP). Following an overdose, APAP induces liver toxicity. Thousands of studies have been written into the mechanisms behind organ damage, cell death, regeneration, and recovery. It is also a very well-liked experimental model to check the effectiveness of several possible medications and chemicals to cure or prevent acute liver damage and encourage regeneration. The APAP overdose model's appeal is largely due to two factors: first, it is clinically relevant, and second, the experimental design is seen to be very straightforward. Regarding therapeutic importance, APAP is a component of dozens of prescription and over-the-counter medications that tens of millions of people worldwide use every day. Even though it's thought to be safe at therapeutic levels, APAP overdoses can induce liver damage that can lead to acute liver failure (ALF) and even patient death.[1,2] APAP is a safe medication at therapeutic dosages of 4 g per day for an adult. According to extensive research evaluations, even vulnerable individuals like alcoholics are unlikely to experience negative effects from therapeutic dosages of APAP.[3] APAP toxicity is the most frequent cause of ALF in the US, the UK, and many other western countries [4] To better understand the mechanism of APAP hepatotoxicity in patients and to create biomarkers that can determine whether a patient will recover on their own or require a liver transplant to survive, this review will concentrate on current developments in these fields.

THE CAUSES OF APAP LIVER DAMAGE: CLINICAL ELEMENTS OF AN OVERDOSE OF APAP:

A therapeutic dosage of APAP for an adult is 4 g once a day. Numerous analyses of the literature indicate that therapeutic dosages of APAP are unlikely to have negative effects on even vulnerable individuals like alcoholics. [2] However, an overdose can cause severe liver injury and even acute liver failure [3,4] A single, severe overdose of APAP is frequently caused by suicide attempts, but accidental, cumulative overdosing is a growing issue. APAP can be found in various over-the-counter pharmaceuticals, including sleeping pills, cold treatments, and a variety of different painkillers. As a result, patients who are taking various medications may unintentionally take more APAP than is advised. Because these individuals usually appear later than those who attempt suicide, unintentional overdose frequently results in a worse prognosis.[3,4] The only clinically approved antidote against APAP-induced liver injury is N-acetylcysteine (NAC), which is most effective when administered within 8 h of

the overdose [1] Even after 24 hours, NAC is still helpful, although its effectiveness has significantly decreased.[6]The reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), a precursor for glutathione (GSH) production, are scavenged by NAC which has this effect, during the metabolism phase.[7] Later on, GSH helps mitochondria scavenge reactive oxygen, and extra NAC is converted to Krebs cycle intermediates to enhance mitochondrial energy metabolism. [6]. Delayed treatment with NAC increases the risk of acute liver failure. However, in contrast to idiosyncratic drug toxicity, APAP-induced liver injury and liver failure have a relatively high survival rate. Nevertheless, due to the drug's widespread use, APAP overdose is by far the most frequent cause of acute liver failure of any etiology in the USA and the UK [8].

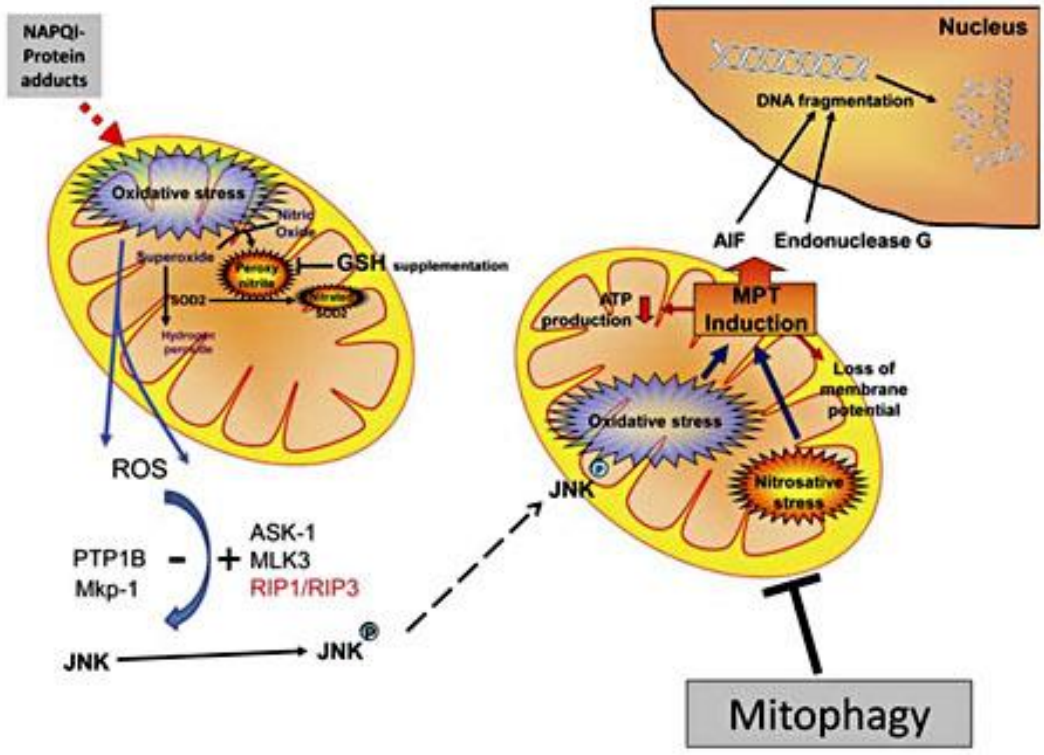
Therefore, the two main difficulties associated with APAP overdose are:

(1) Enhancing our comprehension of the processes of toxicity and regeneration to create therapeutic approaches that lessen the severity of the illness and prevent acute liver failure (2) identifying biomarkers to identify which patients are most likely to recover and which will require a transplant to survive as soon as possible after admission.

Preclinical Studies on the Mechanisms of Liver Damage Caused by APAP

A mouse model that demonstrated a level of liver damage comparable to that seen in people was created soon after the initial publication on the hepatotoxicity of APAP in humans. Early studies showed that a reactive metabolite that can be scavenged by GSH, later identified as NAPQI, forms. NAPQI overproduction following an overdose results in protein adduct formation and toxicity due to interactions with protein sulfhydryl groups. NAC was created as a result of this early understanding of the mechanism.[9] Due to the inability to identify any crucial targets, subsequent investigations showed that the creation of APAP protein adducts does not, in and of itself, immediately result in cell death.[5]Alternatively, protein adducts, particularly in mitochondria, appear to disrupt the electron transport chain and cause oxidative stress as well as the production of peroxynitrite in mitochondria[10] which eventually triggers the mitochondrial permeability transition (MPT) pore opening and collapse of the membrane potential [12] (fig. 1). Additionally, Bax pore formation and subsequent mitochondrial matrix swelling cause the release of intermembrane proteins, particularly endonuclease G and apoptosis-inducing factor, both of which move to the nucleus and significantly fragment DNA.[9,10] Cellular necrosis is caused by the interaction of severe mitochondrial failure and nuclear fragmentation.[12]

APAP-induced necrotic cell death mechanisms. The reactive intermediate NAPQI is produced during the P450-dependent metabolism of APAP and causes the development of mitochondrial protein adducts and oxidative stress. Nitric oxide and superoxide combine to form peroxynitrite, which is a product of the mitochondrial electron transport chain. Superoxide dismutase 2 (SOD2) may break down superoxide and convert it to hydrogen peroxide, however, peroxynitrite can deactivate SOD2 through nitration. The initial oxidative stress in the mitochondria can also activate the MAP kinase JNK through a variety of redox-sensitive kinase pathways, leading to its activation and translocation to the mitochondria. Apoptosis-inducing factor (AIF) and endonuclease are two mitochondrial proteins transported when JNK is translocated within the cell's mitochondria, enhancing the oxidative stress within the cell. [10]



Fig; 1

It was discovered subsequently that the first oxidative stress wasn't enough to start the MPT. Instead, an amplification loop that involves the activation of many redox-sensitive MAP kinases, such as mixed-lineage kinase 3 and apoptotic signal-regulating kinase 1, is required [13]. This leads to the activation of JNK and the translocation of p-JNK to mitochondria. (Fig.1) On coming into contact with an anchor protein in the outer mitochondrial membrane, p-JNK [15]. The MPT is activated by lysosomal iron and oxidative stress together through

the Ca2+ uniporter [14]. Therefore, the JNK loop is essential for the pathophysiology in the mouse model. Recent studies on APAP hepatotoxicity have demonstrated the significance of other kinases, including receptor-interacting protein kinase (RIP) 1 and RIP3 [11,14] It is yet unknown if these kinases function via JNK or another route. It has been demonstrated that RIP3 increases oxidative stress and mitochondrial fission [16]. All signaling mechanisms eventually result in cell necrosis (necroptosis) [12,16]. There is no indication of caspase activation, and very strong caspase inhibitors have little influence on the injury. Therefore, apoptosis is not a key cell death mechanism of APAP-induced liver injury [12,18,20]. Adaptive defense systems and pro-cell death signaling pathways both influence the pathophysiology. Damaged organelles are encased in lipid membranes (autophagosomes) as part of the autophagic process, which then fuses them with lysosomes for destruction [21]. A high APAP dosage triggers damaged mitochondria's autophagy, or mitophagy, which can reduce liver damage [25]. When the damage is not too severe, cell death can be affected by mitophagy near the periphery of necrosis. [24].

Beyond autophagy, it is possible to trigger the unfolded protein response (ER stress) [23], albeit the pathophysiological significance is still debatable. Additionally, adaptive responses—like the production of heat shock proteins—can be advantageous [22]. The anti-oxidant enzymes, such as glutamate cysteine ligase, which is responsible for the rate-limiting step in the production of hepatic GSH. It is believed that the stronger activation of glutamate-cysteine ligase and the quicker recovery of GSH levels are the key reasons why female mice are less susceptible to an overdose of APAP [26].

Overall, a variety of pro-cell death pathways and adaptive defensive systems must be taken into account for all research.

MECHANISM INVOLVED IN HUMAN LIVER STUDIES:

The majority of the liver damage caused by APAP was first identified in a mouse model, but it's crucial to consider whether or not these pathways apply to human patients. Primary human hepatocytes or metabolically capable hepatoma cells (HepaRG cells) respond to high APAP levels by rapidly depleting GSH and forming protein adducts, including mitochondrial adducts. This initial reaction is similar to that of mice hepatocytes [27, 29]. It is now clear that APAP protein adducts occur right away, contrary to the initial hypothesis that GSH levels must first be reduced by around 70% before they start to form [9, 27, 29]. Protein adducts have been seen in mice's liver and plasma even after therapeutic dosages, which only

slightly and momentarily reduce GSH levels [28] in humans [32]. Human hepatocytes generate more protein adducts overall than those seen in rodents, and the development of mitochondrial adducts is thought to occur more slowly in humans than in rodents [27] compared to rodents [31,32]. The collapse of the mitochondrial membrane potential, which is a sign of oxidative stress and mitochondrial malfunction, is followed by cell content release or propidium iodide absorption, which indicates necrotic cell death [27, 29]. Additionally, APAP led to JNK activation in the cytoplasm of primary human hepatocytes by 6 hours, and p-JNK translocation to mitochondria by 15 hours [27]. On the other hand, mice experience JNK activation and mitochondrial p-JNK translocation within 1-2 hours [15.30]. Even though mitochondrial malfunction and oxidative stress are key components of this cell type's cell death process [29], APAP did not activate JNK in HepaRG cells [27]. Compared to mouse hepatocytes, primary human hepatocytes were only marginally decreased in cell mortality by a JNK inhibitor, indicating that human cells are less dependent on the JNK amplification loop [30].

Evidence for mitochondrial dysfunction was also seen in APAP overdose patients, where the release of biomarkers for mitochondrial damage, such as mitochondrial DNA (mtDNA), the mitochondrial matrix enzyme glutamate dehydrogenase, and the indirect mitochondrial damage biomarker nuclear DNA (nDNA) fragments, could be detected in plasma. These findings support the findings with primary cells [4]. Although alanine aminotransferase (ALT) is produced and severe necrosis develops in mice following a furosemide overdose, these biomarkers are not released, indicating that they are selective for mitochondrial injury rather than merely cell death [4]. Patients who have more mitochondrial damage have a worse chance of surviving, proving that mitochondrial dysfunction is essential to the processes of APAP hepatotoxicity in humans [34].

COMPARISON OF INNATE IMMUNE RESPONSE ON HEPATOTOXICITY AMONG: RODENTS AND HUMANS

Damage-associated molecular patterns (DAMPs), such as HMGB1, mtDNA and nDNA fragments, etc., are released as a result of necrotic cell death, as shown in mice and humans following APAP overdose [36,37]. DAMPs cause the production of proinflammatory cytokines and chemokines by binding to several Toll-like receptors, particularly those on macrophages. Interleukin-1 (IL-1) has drawn particular interest because, in addition to transcriptional activation of the gene, a posttranslational cleavage by caspase-1 is required for

its production [37]. The Nod-like receptor family's pyrin domain-containing 3 (NLRP3) inflammasome and several other scaffolding proteins are needed to help activate caspase-1. The NLRP3 inflammasome can be activated by the purinergic receptor P2X7 when it is triggered by DAMPs such as adenosine triphosphate [37]even though there is enough proof of inflammasome activation and the production of IL-1 following APAP overdose in mice [18,38]. It is debatable whether this mediator and the inflammasome have any bearing on pathophysiology [18,39]. The major way that IL-1 may affect APAP-induced liver damage would be to stimulate inflammatory cells like neutrophils because IL-1 receptors cannot directly cause cell death. Although neutrophils are attracted to the liver during the injury phase of APAP hepatotoxicity, several anti-neutrophil therapies have not been successful in reducing liver damage [36]. Due to off-target effects, the findings of a few studies that claimed neutrophils aggravated APAP-induced liver injury need to be questioned [36]. The discovery that circulating and liver-derived neutrophils are not activated during the first stage of liver damage are some of the most persuasive evidence opposing a function for neutrophils [40].

CONCLUSION:

Acute liver failure and drug-induced liver damage are most frequently caused by APAP overdose in Western nations. The processes, which hold for both the mouse model and human pathology, include the production of a reactive metabolite that depletes GSH and causes mitochondrial malfunction with oxidative stress, JNK activation, nDNA fragmentation, and the MPT, which leads to cellular necrosis. Based on these pathways, the medical countermeasure NAC was created.

Even while NAC is quite effective when administered within 8 hours after the overdose, its effectiveness is reduced when therapy is postponed. To find treatment targets that would help patients who appear late, further research into the latter stages of damage and the shift to regeneration is required. Additionally, more and more biomarkers are being found that aid in the comprehension of the mechanisms behind liver injury in patients and, potentially, enable admission-time prediction of whether a patient will recover or require a liver transplant to survive.

REFERENCES:

1. Larson, A. M. Acetaminophen hepatotoxicity. Clin. Liver Dis. 2007, 11, 525–548.

2. Dart R C, Bailey E: Does therapeutic use of acetaminophen cause acute liver failure? Pharmacotherapy 2007; 27:1219-1230.
3. Fontana R J: Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspectives. Gastroenterology 2014; 146:914-928.
4. Mc Gill M R, Sharpe M R, Williams CD, et al., The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest 2012; 122:1574-1583.
5. Smilkstein M J, Knapp G L, Kulig K W, Rumack B H: Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. N Engl J Med 1988; 319:1557-1562.
6. Saito C, Zwingmann C, Jaeschke H: Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. Hepatology 2010; 51:246-254.
7. Corcoran G B, Racz W J, Smith C V, Mitchell JR: Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther1985; 232:864-872.
8. Lee WM: Acute liver failure. Semin Respir Crit Care Med 2012; 33:36-45.
9. Mitchell J R, Jollow D J, Potter W Z, Gillette J R, Brodie B B: Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther1973; 187:211-217.
10. Jaeschke H, McGill M R, Ramachandran A: Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev 2012; 44:88-106.
11. Kon K, Kim J S, Uchiyama A, Jaeschke H, Lemasters J J: Lysosomal iron mobilization and induction of the mitochondrial permeability transition in acetaminophen-induced toxicity to mouse hepatocytes. Toxicol Sci 2010; 117:101-108.
12. Gujral J S, Knight T R, Farhood A, Bajt M L, Jaeschke H: Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? Toxicol Sci 2002; 67:322-328.
13. Han D, Dara L, Win S, Than T A, Yuan L, et al., Regulation of drug-induced liver injury by signal transduction pathways: critical role of mitochondria. Trends Pharmacol Sci 2013; 34:243-253.
14. Saito C, Lemasters J J, Jaeschke H: c-Jun N-terminal kinase modulates oxidant stress and peroxynitrite formation independent of inducible nitric oxide synthase in acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2010; 246:8.
15. Han D, Dara L, Win S, Than T A, Yuan L, et al., Regulation of drug-induced liver injury by signal transduction pathways: critical role of mitochondria. Trends Pharmacol Sci 2013; 34:243-253.
16. Ramachandran A, McGill M R, Xie Y, Ni HM, et al., Receptor interacting protein kinase 3 is a critical early mediator of acetaminophen-induced hepatocyte necrosis in mice. Hepatology 2013; 58:2099-2108.
17. Ding W X, Yin X M: Mitophagy: mechanisms, pathophysiological roles, and analysis. Biol Chem 2012; 393:547-564.
18. Jaeschke H, Cover C, Bajt M L: Role of caspases in acetaminophen-induced liver injury. Life Sci 2006; 78:1670-1676.
19. Williams C D, Farhood A, Jaeschke H: Role of caspase-1 and interleukin-1β in acetaminophen-induced hepatic inflammation and liver injury. Toxicol Appl Pharmacol2010; 247:169-178.
20. Zhang Y F, He W, Zhang C, Liu X J, Lu Y, Wang H, Zhang Z H, Chen X, Xu D X: Role of receptor-interacting protein (RIP)1 on apoptosis-inducing factor-mediated necroptosis during acetaminophen-evoked acute liver failure in mice. Toxicol Lett 2014; 225:445-453.
21. Jaeschke H, Cover C, Bajt M L: Role of caspases in acetaminophen-induced liver injury. Life Sci 2006; 78:1670-1676.
22. Ding W X, Yin X M: Mitophagy: mechanisms, pathophysiological roles, and analysis. Biol Chem 2012; 393:547-564.
23. Tolson J K, Dix D J, Voellmy R W, and Roberts SM: Increased hepatotoxicity of acetaminophen in Hsp70i knockout mice. Toxicol Appl Pharmacol 2006; 210:157-162.
24. Nagy G, Kardon T, Wunderlich L, Szarka A, Kiss A, et. al., Acetaminophen induces ER-dependent signaling in mouse liver. Arch Biochem Biophys2007; 459:273-279.
25. Ni H M, Williams J A, Jaeschke H, Ding W X: Zonated induction of autophagy and mitochondrial spheroids limits acetaminophen-induced necrosis in the liver. Redox Biol 2013; 1:427-432.

26. Ni H M, Bockus A, Boggess N, Jaeschke H, and Ding W X: Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatology 2012; 55:222-232.
27. Du K, David Williams C, McGill M R, Jaeschke H: Lower susceptibility of female mice to acetaminophen hepatotoxicity: role of mitochondrial glutathione, oxidant stress, and c-jun N-terminal kinase. Toxicol Appl Pharmacol2014; 218:58-66.
28. Xie Y, McGill M R, Dorko K, Kumar S C, Schmitt T M, Forster J, Jaeschke H: Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. Toxicol Appl Pharmacol2014; 279:266-274.
29. McGill M R, Yan H M, Ramachandran A, Murray G J, and Rollins D E, Jaeschke H: Hepa R G cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology 2011; 53:974-982.
30. McGill M R, Lebofsky M, Norris H R, Slawson M H, Bajt ML, Xie Y, Williams C D, Wilkins D G, and Rollins D E, Jaeschke H: Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. Toxicol Appl Pharmacol2013; 269:240-249.
31. Saito C, Yan H M, Artigues A, Villar M T, Farhood A, Jaeschke H: Mechanism of protection by metallothionein against acetaminophen hepatotoxicity. Toxicol Appl Pharmacol2010; 242:182-190.
32. McGill M R, Williams C D, Xie Y, and 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. Toxicol Appl Pharmacol 2012; 264:387-394.
33. Heard K J, Green J L, James L P, Judge B S, Zolot L, Rhyee S, Dart R C: Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. BMC Gastroenterol 2011; 11:20.
34. McGill M R, Li F, Sharpe M R, Williams C D, Curry S C, and Ma X, Jaeschke H: Circulating acylcarnitines as biomarkers of mitochondrial dysfunction after acetaminophen overdose in mice and humans. Arch Toxicol 2014; 88:391-401.
35. McGill M R, Staggs V S, Sharpe M R, Lee W M, Jaeschke H, Acute Liver Failure Study Group: Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. Hepatology 2014; 60:1336-1345.
36. Chen C, Krausz K W, Shah Y M, Idle J R, and Gonzalez F J: Serum metabolomics reveals irreversible inhibition of fatty acid β-oxidation through the suppression of PPARα activation as a contributing mechanism of acetaminophen-induced hepatotoxicity. Chem Res Toxicol2009;22:699-707.
37. Jaeschke H, Williams C D, Ramachandran A, Bajt ML: Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. Liver Int 2012; 32:8-20.
38. Kubes P, Mehal W Z: Sterile inflammation in the liver. Gastroenterology 2012;143:1158-1172.
39. Imaeda A B, Watanabe A, Sohail M A, Mahmood S, Mohamadnejad M, Sutterwala F S, Flavell R A, Mehal W Z: Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. J Clin Invest 2009; 119:305-314.
40. Williams C D, Antoine D J, Shaw P J, Benson C, Farhood A, Williams DP, Kanneganti T D, Park B K, Jaeschke H: Role of the Nalp3 inflammasome in acetaminophen-induced sterile inflammation and liver injury. Toxicol Appl Pharmacol 2011; 252:289-297.
41. Williams C D, Bajt ML, Farhood A, Jaeschke H: Acetaminophen-induced hepatic neutrophil accumulation and inflammatory liver injury in CD18-deficient mice. Liver Int 2010; 30:1280-1292.

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