Acetaminophen Induced Liver Injury: Metabolism and Inflammation Perspectives

DITTY J MARY¹, EZHILARASAN D²∗

¹Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical & Technical Sciences,
²Assistant Professor, Department of Pharmacology, Saveetha Dental College and Hospitals Saveetha Institute of Medical & Technical Sciences
*Corresponding Author
E- mail: ezhiid@gmail.com

Abstract: Acetaminophen (APAP) overdose is the most common cause of acute liver failure in the US, and decades of intense study of its pathogenesis resulted in the development of the antidote N-acetylcysteine, which facilitates scavenging of the reactive metabolite and is the only treatment in clinical use. However, the narrow therapeutic window of this intervention necessitates a better understanding of the intricacies of APAP-induced liver injury for the development of additional therapeutic approaches that can benefit late-presenting patients. More recent investigations into APAP hepatotoxicity have established the critical role of mitochondrial dysfunction in mediating liver injury as well as clarified mechanisms of APAP-induced hepatocyte cell death facilitates scavenging of the reactive metabolite and is the only treatment in clinical use. Mitochondrial oxidative and nitrosative stress is a key mechanistic feature involved in downstream signaling after APAP overdose. The identification of specific mediators of necrotic cell death further establishes the regulated nature of APAP-induced hepatocyte cell death. In addition, the discovery of the role of mitochondrial dynamics and autophagy in APAP-induced liver injury provides additional insight into the elaborate cell signaling mechanisms involved in the pathogenesis of this important clinical problem. In spite of these new insights into the mechanisms of liver injury, significant controversy still exists on the role of innate immunity in APAP-induced hepatotoxicity.

Keywords: Acetaminophen toxicity, Hepatotoxicity, Acute liver failure (ALF)

INTRODUCTION

Acetaminophen (APAP) is one of the most common analgesic and antipyretic drugs in use globally. (Lee, 2008) Though the drug is safe and effective at therapeutic doses. (Anitha and Ashwini, 2017) The therapeutic window is narrow, and an overdose is highly hepatotoxic. (Larson et al., 2005) Because of the ubiquitous nature and broad availability of the drug has resulted in APAP induced hepatotoxicity being the most frequent cause of acute liver failure (ALF) in the US and other Western countries (Ashwini, Ezhillaras and Anitha, 2017) (Larson et al., 2005) Acetaminophen induced hepatotoxicity remains as a significant public health concern and common indication for emergent liver transplantation as the most common cause of ALF in the USA and UK. (Bateman et al., 2014) (Lakshmi et al., 2015) Acetaminophen combination products frequently prescribed by physicians and other healthcare professionals, with unintentional and chronic overdose accounting for over 50% of cases of APAP-related ALF. (Lancaster, Hiatt and Zarrinpar, 2015; Sharma et al., 2019) APAP hepatotoxicity remains a global issue; it accounts for more than 50% of overdose-related acute liver failure in the united states and approximately 20% of the liver transplant cases. (Nelson, 1990; Yoon et al., 2016) The pathophysiology of acute liver failure secondary to APAP toxicity remains to be precisely elucidated, and has adverse patient outcomes such as increased morbidity and mortality. (Ezhilarasan, Lakshmi, Vijayaragavan, et al., 2017) (Xie et al., 2015) Decades of investigations into the mechanisms of APAP-induced liver injury have provided significant insight into the role of APAP metabolism and formation of a reactive metabolite in initiating the cascade of events ultimately leading to liver injury. (Court et al., 2017) Hepatic injury and subsequent hepatic failure due to both intentional and unintentional overdose of acetaminophen (APAP) has affected patients for decades, and involves the cornerstone metabolic pathways which take place in the microsomes within hepatocytes. (McGill and Jaeschke, 2013) Oxidative stress, inflammation, mitochondrial dysfunction and hepatocytes death have been attributed to APAP intoxication in the liver. (Lancaster, Hiatt and Zarrinpar, 2015) (Perumalsamy et al., 2018). Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Deogade, Gupta and Ariga, 2018; Ezhillaras, 2018; Ezhillaras, Sokal and Najimi, 2018; Jeevanandan and Govindaraju, 2018; J et al., 2018; Menon et al., 2018; Prabakar et al., 2018;

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Metabolism of Acetaminophen

Acetaminophen is extensively metabolized by the liver via three main hepatic pathways: glucuronidation, sulfation, and CYP450 2E1 oxidation. Approximately 90% of acetaminophen is conjugated to sulfated and glucuronidated metabolites that are readily eliminated. Acetaminophen metabolism occurs in the hepatocytes (Mehta et al., 2019). At therapeutic doses, APAP (90%) undergo glucuronic acid or sulfate conjugation and is excreted through the kidneys (Myers et al., 1995; Ezharasar, Lakshmi, Nagaei, et al., 2017). A minor component is acted upon by cytochrome P450 enzymes such as Cyp2E1 and Cyp1A2 to form a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Matthews, 1997). Though highly reactive, NAPQI is rarely harmful after consumption of therapeutic doses because it is rapidly conjugated with abundant intracellular antioxidants such as glutathione stores in the hepatocytes and excreted through the bile (Hadi et al., 2013; Ezharasar, 2018). However, this contrasts to the scenario after consumption of an overdose of APAP, where the sulfation pathway is saturated and NAPQI generation is significantly elevated in spite of the high capacity of the glucuronidation pathway (Qiu, Benet and Burlingame, 1998; McGill and Jaeschke, 2013). This results in robust reaction with hepatic glutathione stores and the subsequent rapid depletion of glutathione stores in the hepatocytes of the liver (Andringa et al., 2008). Metabolism of APAP can be influenced by genotype differences, and variations in glucuronidation are seen in different populations due to polymorphisms in the UDP-glucuronosyltransferase (UGT) enzymes (Bruderer et al., 2015). N-acetylcysteine is reported to facilitate scavenging of APAP derived reactive metabolites and is the only treatment for APAP overdose (Wong et al., 2020). The mechanism of APAP metabolism associated with changes in liver is presented in Figure 1.

Acetaminophen Induced Liver Damage

Hepatic injury and subsequent hepatic failure due to both intentional and unintentional overdose of acetaminophen (APAP) has affected patients for decades, and involves the cornerstone metabolic pathways which take place in the microsomes within hepatocytes. Acetaminophen-induced hepatotoxicity remains a significant public health concern and is a common indication for emergent liver transplantation (Jaeschke, 2003). (Moalem, 2020) APAP associated ALF cases have non-specific symptoms such as nausea, vomiting, and malaise (Ezharasar, 2018; Gheena and Ezharasar, 2019b). Severe acute liver injury often leads to impaired elimination of bilirubin, manifesting as jaundice immediately prior to or shortly after presentation. In addition, the depressed synthesis and excessive consumption of clotting factors results in a complex coagulopathy (McGill et al., 2012). A diminished synthesis of glucose, increased intracellular lactate production, and the reduction in hepatic uptake of lactate leads to hypoglycemia, and metabolic acidosis (Menon et al., 2018). Thirty to forty percent of ALF patients present with impaired renal function and associated azotemia and oliguria (Ring-Larsen and Palazzo, 1981). (Jaeschke, 2015) Liver damage from APAP can be severe, which can result either from an overdose or from regular doses that are taken while drinking alcohol (Bruderer et al., 2015). Most of APAP induced liver injury cases are caused by an intentional or suicidal overdose. (Cooper et al., 2009) Oxidative stress mediated by hepatotoxic APAP metabolite N-acetyl-p-benzoquinoneimine (NAPQI), is considered as the main cause of hepatocellular degeneration. (Murray et al., 2008). APAP causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. (Court et al., 2017). APAP protein adducts that form upon NAPQI conjugation with protein sulfhydryl groups of cysteine in GSH or cellular proteins readily interact with mitochondria (Holt, Cheng and Ju, 2008). These mitochondrial protein adducts are thought to cause mitochondrial dysfunction and to promote oxidative stress. (Xie et al., 2014). (Yang et al., 2015) Therefore, detection of elevated levels of these APAP-protein adducts can be used for clinical indication of APAP hepatotoxicity. (Antoine et al., 2015).

Other mechanisms of hepatotoxicity induced by APAP include the formation of toxic free radicals, such as peroxynitrite, from the reaction of superoxide and nitric oxide, subsequently forming nitrotyrosine adducts inside the mitochondria (Jaeschke et al., 2012). GSH repletion not only provides surplus cysteine as an energy substrate for the Krebs cycle, it also serves the important role of scavenging for free radicals and peroxynitrite (Menon et al., 2018; Rajeshkumar, Kumar, et al., 2018). Mitochondria, are critical for cellular respiration and the metabolism, and suffers damage to their own mitochondrial DNA by the actions of ROS and peroxynitrite compounds, and they have been directly implicated in the process leading to cessation of ATP synthesis (Liu, Govindarajan and Kaplowitz, 2004). The detection of one or more biomarkers in the setting of APAP hepatotoxicity is desirable, especially in clinical scenarios, the diagnosis of APAP hepatotoxicity is

Acetaminophen Induced Hepatic Inflammation

Acetaminophen overdose can result in serious liver injury and potentially death. APAP induced hepatotoxicity mainly depends on metabolism of APAP to a reactive metabolite initiating a cascade of intracellular events resulting in hepatocellular necrosis. (Leang et al., 2014) (Rajeshkumar, Agarwal, et al., 2018) An early APAP injury triggers sterile inflammatory response with formation of cytokines and innate immune cell infiltration in the liver. (Lee, 2004) The extensive hepatocellular necrosis after an APAP overdose leads to release of damage-associated molecular patterns (DAMPs) including mitochondrial DNA, nuclear DNA fragments, high-mobility group box (HMGB1) protein, and many others. (Hawton, 2001) DAMPs bind to pattern recognition receptors such as toll-like receptors (TLRs) on inflammatory cells and transcriptionally activate cytokine formation in inflammatory cells (Martin-Murphy, Holt and Ju, 2010). During APAP intoxication, inflammatory cells in the liver produce several proinflammatory markers which further aggravates liver inflammation. Diet restriction inhibits apoptosis and HMGB1 oxidation which promotes the inflammatory cell recruitment during acetaminophen hepatotoxicity. The role of damage is associated with the molecular pattern of molecules in acetaminophen-induced liver injury in mice. (Bunchorntavakul and Reddy, 2013) Due to some unique properties, IL-18 stands out among members of the IL-1 cytokine family. (Sandhu and Navarro, 2020) IL-18 is constitutively expressed in a variety of cell types, for example, in hepatic Kupffer cells Accordingly, IL-18 expression is detectable in healthy murine liver where macrophages/Kupffer cells are a major source of bioactive IL-18. (Mühl and Pfleischfritter, 2004). (Hayashi et al., 2007) The DAMPs release is required for the APAP-induced sterile inflammatory response. Previous experimental studies reported the release of DAMPs such as HMGB1, heat shock proteins, and DNA fragments in plasma after APAP administration in experimental animals (Sandhu and Navarro, 2020). During APAP induced-necrosis, the necrotic cells passively release hypo-acetylated HMGB1 and macrophages or monocytes actively secretes hyper-acetylated form of HMGB1. (James et al., 2007) (Khandelwal et al., 2011) Our institution is passionate about high quality evidence based research and has excelled in various fields (Pc, Marimuthu and Devados, 2018; Ramesh et al., 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai et al., 2019; Sridharan et al., 2019; Vijayashree Priyadharsini, 2019; Chandrasekar et al., 2020; Mathew et al., 2020; R et al., 2020; Samuel, 2021)

Fig.1: Mechanisms of acetaminophen (APAP) metabolism associated liver injury. LPO, lipid peroxidation; M, mitochondria; NAPQI, N-acetyl-p-benzoquinoneimine; GSH, reduced glutathione; IL, interleukin; TNF, tissue necrosis factor alpha; KC, Kupffer cells; DAMPs, damage-associated molecular patterns; ROS, reactive oxygen species; MAPK, mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; N, nucleus
CONCLUSION
Over the last several decades, significant progress has been made in the understanding of the intracellular signaling mechanisms leading to APAP-induced cell death in hepatocytes in experimental animals and humans. Although more can be learned about various aspects of these mechanisms, it is important to keep in mind the potential effects of intervention strategies on drug metabolism, which can lead to misinterpretations. It is also critical to connect any newly discovered mediators and pathways to the established mechanisms. The recent studies have explored the role of the immune system, DAMPs and inflammasome in APAP hepatotoxicity. Therefore, future experimental and clinical studies can target APAP-induced liver injury by employing novel anti-inflammatory and immunomodulatory agents.

Source of Support
Nil

CONFLICT OF INTEREST
None Declared

Abbreviations
- APAP- Acetaminophen
- ALF- Acute liver failure
- DAMP- damage-associated molecular pattern
- GDCA- glycodeoxycholic acid
- GDH- glutamate dehydrogenase
- GSH- glutathione
- miRNA- microRNA
- mtDNA- mitochondrial DNA
- NAC-N-acetyl cysteine
- NAPQI-N-acetyl-para-benzo-quinone imine
- nDNA- nuclear DNA
- ROS- reactive oxygen species
- SULT- sulfotransferase
- UGT- UDP-glucuronosyltransferase

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