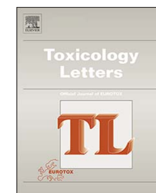
Toxicology Letters 283 (2018) 69–76

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



# Comparison of hepatic transcriptome profiling between acute liver injury and acute liver failure induced by acetaminophen in mice

Chunmin Li[a,1], Yanan Ming[a,1], Wenting Hong[b], Yingyue Tang[a], Xiaohong Lei[a], Xiaobo Li[b,*], Yimin Mao[a,**]

[a] *Division of Gastroenterology and Hepatology, Key Laboratory of Gastroenterology and Hepatology, Ministry of Health, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Institute of Digestive Disease, Shanghai, China*
[b] *Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Fudan University, Shanghai, China*

ARTICLE INFO

*Keywords:*
Acetaminophen
Liver injury
Liver failure
Transcriptome
Biomarkers

ABSTRACT

Acetaminophen (APAP) overdose is a leading cause of drug-induced acute liver failure in many countries. In the present study, we developed stable mouse models of acute drug-induced hepatic injury (DILI) and acute drug-induced hepatic failure (DILF) by sub-lethal and lethal APAP injection respectively. The differences in hepatic transcriptome profiling between these two models were compared by RNA sequencing, which were validated by qPCR, western-blot and ELISA. In results, serum IL-6, TNF-a and IL-10 levels are higher in DILF than in DILI. The upregulated genes in DILF compared with DILI were mostly enriched in the areas of "cellular development process", "cell division", "multicellular organism development," etc. The downregulated genes in DILF compared with DILI were mostly enriched in the areas of "cellular response to chemical stimulus", "cellular response to stress", "cell activation," etc. Sub-lethal doses of APAP increased Myc, Bag3 and Btc expression in mouse liver, but lethal doses of APAP did not, which suggested that these three genes might play important roles in adaptive protection reactions in DILI. The serum Btc level might be a potential biomarker of drug induced liver injury with good prognosis. Our data can help us better understand the mechanisms of hepatotoxicity that influence prognosis and seek novel prognostic indicators of DILI.

## 1. Introduction

Drug-induced liver injury (DILI) has been a major problem that currently threatens human health and drug development, and has been a major cause of acute liver failure in the U.S. and some western countries(Holubek et al., 2006; Lancaster et al., 2015). Acetaminophen (N-acetyl-*p*-aminophenol, APAP) has been widely used as an analgesic and antipyretic drug that is known to be safe at the therapeutic dose. However, acute liver injury caused by APAP overdose or when misused in at-risk populations has been the main cause of DILI in many countries (Lee et al., 2008).

It is generally assumed that APAP-induced hepatotoxicity is related to the formation of a reactive metabolite, N-acetyl-*p*-benzoquinone imine (NAPQI). Excessive NAPQI can be detoxified by conjugation with cellular reduced glutathione (GSH) in the liver(Yuan and Kaplowitz, 2013). Subsequently, adducts formed, which might induce oxidative stress, mitochondrial damage, c-jun N-terminal kinase (JNK) activation, and nuclear DNA fragmentation, leading to cell death(Cohen et al., 1997; Jaeschke et al., 2003; McGill et al., 2012a). Liver injury caused by APAP is dose dependent because higher APAP intake is associated with greater NAPQI formation, GSH depletion, covalent binding and oxidative stress. The degree of hepatotoxicity varies from acute mild liver injury to severe and threatening liver failure. The underlying mechanisms of hepatotoxicity, especially different degrees of hepatotoxicity in DILI, are still not fully understood.

It is generally known that biomarkers are obtained from the blood, urine, and many other biological samples that may provide insight into the cause, severity, or outcome of a DILI case(Beger et al., 2015). The serum biomarkers most commonly used to detect acute and chronic liver injury are serum alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total bilirubin levels. Serum ALT is more liver-specific than AST, but it is not aetiology-specific (Green and Flamm, 2002; Sayuk et al., 2007). Serum ALP levels are not liver-specific and may be elevated in other diseases(Beger et al., 2015). Similarly, total bilirubin levels are an insensitive marker for most forms of liver disease. In addition, several studies have

* Corresponding author at: Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Fudan University, Shanghai, China.
** Corresponding author.
*E-mail addresses:* xbli@fudan.edu.cn (X. Li), maoym11968@163.com (Y. Mao).
[1] Authors contribute equally.

https://doi.org/10.1016/j.toxlet.2017.11.020
Received 13 September 2017; Received in revised form 20 November 2017; Accepted 22 November 2017
Available online 23 November 2017
0378-4274/ © 2017 Elsevier B.V. All rights reserved.

demonstrated that serum SDH, GLDH, miR-122, HMGB1, and haemeoxygenase 1 (HMOX1) levels also increased following APAP overdose (Gao et al., 2017; Watkins et al., 2006; Wetmore et al., 2010). A recent study reported that APAP-induced liver injury increases the amount of total and liver-specific proteins in extracellular vesicles (EVs), further supporting the utility of detecting EV proteins as reliable and noninvasive biomarkers of liver injury (Cho et al., 2017). Meanwhile, another report identified the utility of a non-invasive longitudinal imaging approach for providing direct visualization of liver function (Brillant et al., 2017). The dosage and condition of patients can influence the prognosis of the liver damage induced by drugs, including APAP. How to determine the prognosis of DILI is a problem that demands prompt solution. However, none of the indicators that are used presently have been able to solve the issue (Beger et al., 2015; Green and Flamm, 2002; Iruzubieta et al., 2015; Sayuk et al., 2007; Watkins et al., 2006; Wetmore et al., 2010).

The minimum dosage of acetaminophen for inducing hepatotoxicity is between 125 and 150 mg/kg (Chun et al., 2009; Gokhale and Martin, 2012). In the present study, we developed a stable animal model of drug-induced acute hepatic injury and drug-induced acute hepatic failure with different doses of acetaminophen (APAP) by intraperitoneal injection. We compared hepatic transcriptome profiling between these two models by RNA sequencing (RNA-seq) and tried to further understand the mechanisms of hepatotoxicity influencing the prognosis and seek novel prognostic indicators of DILI.

## 2. Materials and methods

### 2.1. Animals

C57BL/6 male mice aged 8–10 weeks were purchased from the Shanghai Experimental Animal Centre of the Chinese Academy Sciences (Shanghai, China). All mice were maintained under controlled humidity (50% ± 5%), temperature (24° ± 2 °C) and specific pathogen-free conditions with a 12 h light/dark cycle. Animals had free access to water and standard normal chow (NCD:3.85 kcal/g, 10% fat, 20% protein, 70% carbohydrate, formula D12450B, Research Diets Inc) throughout the experimental period. All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine and were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

### 2.2. Treatment of mice

Acetaminophen (Sigma Aldrich, USA) solution was prepared for each experiment by dissolving the drug in saline. Mice were fasted from 6:00 PM until 10:00 AM the following day, at which time they were intraperitoneally (i.p.) injected with acetaminophen at a dosage of either 300 mg/kg (body weight) or 750 mg/kg (body weight) or with saline. Mice were then sacrificed after APAP intoxication, and the liver tissue and plasma were collected at the indicated time points (0 h, 1 h, 3 h, 6 h, and 12 h, n = 6–7 per time point). Some mice were injected with saline as parallel controls at the same time points (1 h, 3 h, 6 h, and 12 h, n = 6–7 per time point). The livers were frozen at −80 °C for later analysis or fixed in 4% paraformaldehyde overnight for histological examination.

### 2.3. Biochemical assays and histopathology

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected by an automatic biochemical analyser (SIEMENS ADVIA 1800: SIEMENS Healthcare Diagnostics, USA). Liver tissues from saline- or APAP-treated mice were fixed in 4% paraformaldehyde overnight and embedded in paraffin, sliced at 5 mm thickness and stained with haematoxylin and eosin (H&E).

### 2.4. Total RNA extraction and RNA-seq library construction for illumina sequencing

Total RNA was prepared from mouse livers using Trizol reagent (Life Technologies, Thermo Fisher Scientific) according to the manufacturer's protocol. Samples were prepared for sequencing using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, #E7530) to enrich mRNA and prepare cDNA libraries. Library quality was assessed using the Agilent 2100 Bioanalyzer. Sequencing was performed at RiboBio Co., Ltd. (Guangzhou, China), on an Illumina HiSeq 3000.

### 2.5. RNA-seq data analysis and functional analysis

The RNA-seq data were analysed as described previously (Wang et al., 2017). The mouse genome sequence was obtained from the UCSC Genome Browser (mm 10) (http://genome.ucsc.edu/). The annotated gene models (NCBIM38) were taken from Ensembl (http://www.ensembl.org/). The RNA-seq reads from the FASTQ files were mapped to the mouse reference genome (mm 10), and the splice junctions were identified by TopHat. The output files in BAM (binary alignment/map) format were analysed by Cufflinks to estimate the transcript abundance. The mRNA abundance was expressed in FPKM (fragments per kilobase of exon per million reads mapped). Cuffdiff 2 version 2.0.2 was used to detect differentially (over 1.5-fold and $P < 0.05$) expressed genes between the indicated groups. DAVID (http://david.abcc.ncifcrf.gov/home.jsp) functional annotation cluster analysis was performed on the list of differentially expressed genes. Only terms for which $P < 0.05$ were selected for analysis. The gene ontology (GO) biological process (BP) terms in DAVID were used to categorize the enriched biological themes in the list of differentially expressed genes.

### 2.6. Quantitative real-time PCR

Total RNA was extracted from the mouse livers using Trizol reagent (Life Technologies, Thermo Fisher Scientific). Reverse transcription (RT) was performed using PrimeScript RT Reagent Kit (Takara, Shiga, Japan), according to the manufacturer's instructions. Relative expressions of the target genes were determined by SYBR Premix Ex Taq II (Takara, Shiga, Japan). Quantitation of the tested genes was carried out using the comparative cycle threshold (CT) method, and the results were normalized to mouse 36B4. Sequences of the primers used for gene expression analysis in this study are shown in Table 1.

### 2.7. Western blot analysis

Proteins from the treated livers were extracted in radioimmunoprecipitation assay lysis buffer (Beyotime, China). Total protein samples of 60 μg each were separated on a 12% SDS-polyacrylamide gel and electro-transferred to PVDF membranes. After blocking with 5% skimmed milk in a mixture of tris–buffered saline and Tween 20 (TBST) for 1 h at room temperature, the membranes were incubated with rabbit polyclonal antibodies against mouse Bag3 (Proteintech) overnight at 4 °C. GAPDH was used as a loading control. The protein signals

**Table 1**
Primers sets used for qPCR.

| Gene name | Prime (5′–3′) | Accession Number |
|---|---|---|
| 36b4 | Forward: TGAGATTCGGGATATGCTGTTGG<br>Reverse: CGGGTCCTAGACCAGTGTTCT | NM_007475.5 |
| Myc | Forward: ATGCCCCTCAACGTGAACTTC<br>Reverse: GTCGCAGATGAAATAGGGCTG | NM_001177352.1 |
| Bag3 | Forward: AGCTCCGACCAGGCTACATT<br>Reverse: GAGTAGGCATGGAAAAGGTGG | NM_013863.5 |
| Btc | Forward: GAAAACCCACTTCTCTCGGTG<br>Reverse: GCAGGAGGGAGTTTGCTCG | NM_007568.5 |

Download English Version:

https://daneshyari.com/en/article/8553514

Download Persian Version:

https://daneshyari.com/article/8553514

Daneshyari.com