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Acetaminophen-induced liver injury: Implications for temporal homeostasis of lipid metabolism and eicosanoid signaling pathway

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ABSTRACT

Acetaminophen is a commonly used drug that induces serious hepatotoxicity when overdosed, leading to increased levels of serum aminotransferases. However, little knowledge exists linking acetaminophen to liver free fatty acids and the eicosanoid-mediated signaling pathway. To this end, adult NMRI mice injected with a dose of 400 mg/kg acetaminophen were monitored for one week post-treatment. Consistent changes were observed in serum transaminases, profile of hepatic free fatty acids, expression of cyclooxygenase, elongase, lipogenesis, and lipolysis genes; as well as in expression patterns of cyclooxygenase-1 and -2 in the liver. Both linoleic acid and arachidonic acid – substrates in eicosanoid biosynthesis – were significantly influenced by overdose, and the latter peaked first among the free fatty acids examined here. There was a close similarity between the temporal dynamics of linoleic acid and aspartate aminotransferases. Moreover, serum transaminases were reduced by cyclooxygenase-2 inhibitors, but not by cyclooxygenase-1 inhibitors. Our results hence attest to the hazard of acetaminophen overdose on the temporal homeostasis of hepatic concentrations of free fatty acids and expression of key genes underlying liver lipid metabolism. There is also evidence for activation of a cyclooxygenase-mediated signaling pathway, especially the cyclooxygenase 2-prostanoid pathway, during acetaminophen-induced liver injury. Therefore, the results of the present study should provide valuable information to a wide audience, working to understand the health hazard of this drug and the implications of the eicosanoid signaling pathway in liver pathophysiology.

1. Introduction

Acetaminophen (*N*-acetyl-p-aminophenol, abbreviated as APAP) is one of the most widely used analgesic, antipyretic, and anti-inflammatory drugs [1]. While generally considered to be safe for humans at a maximum recommended daily dose of 4000 mg (mg) per healthy adult [2], at higher concentrations this drug can cause serious adverse effects, especially toxicity to the gastrointestinal, renal, and hepatic systems [1]. Moreover, APAP poisoning, either by accidental or intentional overdose, is a frequent problem in current medical practice. For example, it is the paramount cause of acute liver failure in Western Europe, the United States, Canada, and Australia [3]. Therefore, an extensive body of literature dealing with acetaminophen hepatotoxicity has emerged over the past few decades. However, the long-term hazard of this drug on liver functions is still far from being completely understood, and even less so is the extent to which it interferes with the temporal dynamics of hepatic lipid metabolism.

Abbreviations: APAP, acetaminophen; ELOVL, elongases; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAB, 3,3′-Diaminobenzidine; PBS, phosphate buffered saline; GC–MS, gas chromatography-mass spectrometry.

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Liver cells are involved in many pathways of lipid metabolism, such as oxidizing triglycerides to produce energy, lipoprotein production, conversion of excess carbohydrates and proteins into fatty acids and triglyceride, or synthesis of cholesterol and phospholipids [4–6]. Acetaminophen has been demonstrated to irreversibly inhibit fatty acid β-oxidation [7], disrupt lipid metabolism and increase triglyceride levels in the serum and liver [7–9]. These metabolic changes perturb hepatocellular functions since affected hepatocytes begin to accumulate intracellular lipids, thus initiating the pathway leading to steatosis – a hallmark of liver damage [7,10]. In fact, cardiovascular diseases, chronic alcohol consumption, chronic exposure to toxic compounds, and many other conditions are associated with liver steatosis [7]. However, such studies have addressed the impact of this drug on hepatic lipid metabolism only for short periods of time after exposure (several hours), and have not investigated its impact on the temporal dynamics of free fatty acid content and key gene expression in the liver although this organ is central to lipid metabolism [10].

Polyunsaturated fatty acids, primarily arachidonic acid, are the precursors of inflammatory metabolites such as eicosanoids. Linoleic acid is an additional source of arachidonic acid, and therefore, this compound also serves as a key player in eicosanoid biosynthesis [11,12]. The cyclooxygenase enzymes convert arachidonic acid via the cyclic pathway of eicosanoid biosynthesis to prostaglandin H2, which is further metabolized to prostaglandins (e.g., PGE2, PGF2α, PGD2, PGI2) and thromboxane; many of which exhibit pro-inflammatory properties [13,14]. There are three types of cyclooxygenases, sharing 60% of their primary structure: COX-1, which is generally localized in the endoplasmic reticulum; COX-2, which is found on the nuclear envelope [14,15]; and COX-3, which occurs only in the cerebral cortex [16]. In the healthy liver, COX-1 and COX-2 work together to induce an inflammatory state, and when needed, to reinstate normal hepatic function [17,18]. At the hepatic level, these cyclooxygenases are abundantly expressed during liver injury, cirrhosis, and induced tumorigenesis [19], suggesting the involvement of eicosanoids in the pathogenic mechanisms of liver injury. They are expressed by the liver macrophage-like Kupfer cells, identified by the macrophage marker ED2 (CD168), and these cells are sites of intense eicosanoid production and signaling [20]. Still, in spite of all these data, little knowledge exists regarding the interplay between APAP treatment and the cyclooxygenase-mediated signaling pathway in the liver. It is known, though, that COX-2 overexpression in the mouse liver induces chronic hepatitis by inducing a persistent inflammatory reaction involving macrophages that causes a persistent increase in the hepatocyte death, which reinforces the inflammatory reaction and thus further death of hepatocytes [21].

In this context, we investigated the effects of acetaminophen on the temporal dynamics of hepatic free fatty acids in the first week post-treatment, and the activation and role of the cyclooxygenase-mediated signaling pathway during this time. We measured serum aspartate and alanine aminotransferase levels as an index of hepatotoxicity [10]. We also assessed the expression patterns of key genes involved in liver lipogenesis and lipolysis. We show here that the hepatic free fatty acid content, as well as the expression of key liver genes, is significantly affected by acute drug overdose, especially during the first days post-treatment. The activity of certain genes did not completely return to the normal range seven days after this toxic event although the serum transaminases did. Moreover, we found an association between the cyclooxygenase-mediated signaling pathway and APAP-induced liver damage. Our results should therefore provide valuable information to a wide array of pharmacologists, clinicians, toxicologists, and biochemists working to understand the hazard of acetaminophen administration and the implications of the eicosanoid-signaling pathway in liver pathophysiology.

2. Materials and methods

2.1. Ethical statement

This study was performed in accordance with the internal guidelines of Victor Babes University of Medicine and Pharmacy from Timisoara, Romania (UMFT). These guidelines comply with the national and European recommendations concerning the protection and welfare of laboratory animals, including those established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [22]. The Ethical Committee of UMFT approved the experimental protocol. All chemicals used in this study were purchased from Sigma–Aldrich, unless otherwise stated.

2.2. Experimental design and mouse rearing

During the experiment, 50 adult NMRI mice, aged 6–7 weeks and weighing 28–34 g, were reared under controlled laboratory conditions (22–24 °C, 12 h light: 12 h dark cycle) with ad libitum access to food and water. The mice were purchased from the Cantacuzino Institute (Bucharest, Romania). The test animals were injected intraperitoneally with a single APAP overdose of 400 mg per kilogram (mg/kg) dissolved in 1.2 ml phosphate buffered saline (PBS) per mouse. This dose was chosen because it is known to cause severe toxic effects in humans [23]. Eight animals were sacrificed at the start of the experiment (i.e., 0 h) and used as controls.

At 24 h, the mice were split into two groups. The first group contained 32 mice, which were sacrificed at 24 h, 48 h, 72 h, and 168 h (7 days) post-treatment, with eight animals per each time point. Liver fragments were sampled, and then either homogenized for analysis of free fatty acids and RNA extraction, or fixed in 4% paraformaldehyde for immunohistochemistry. Blood samples were also collected for determining transaminase levels. All analyses were performed in triplicate.

The second group consisted of 10 mice, with half of them receiving a dose of 30 mg kg^{-1} meloxicam (a selective COX-2 inhibitor), whereas the others received a similar dose of valeryl salicylate (a selective inhibitor of COX-1). These mice were sacrificed after 24 h. This parallel mini-experiment aimed at providing insight into the role of COX-1 and COX-2 during acetaminophen-induced liver injury. Liver samples were collected for immunohistochemistry, as well as blood samples for measuring transaminase concentrations (triplicate analyses). In order to further examine the possible mechanism of the inhibitory effect of meloxicam on the serum levels of AST and how this is related to the inflammatory reaction involving macrophages, we stained with ED2 the control livers and livers treated with APAP for 48 h.

2.3. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assays

The blood was collected by cardiac puncture immediately after the mice were sacrificed. The ALT and AST enzymes were analyzed in the serum using the Siemens Diagnostics ALT-AST Kit according to the manufacturer's directions.

2.4. Immunohistochemistry

The liver fragments were fixed overnight in 4% paraformaldehyde, embedded in paraffin, sectioned at a 4 μm thickness, and then mounted on silanized glass slides. LSAB2 kits with DAB as the chromogen (Dako A/S (Glostrup, Denmark)) were used for immunochemical detection of APAP-induced cellular damage. Following deparaffinization and rehydration, the slides were