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Review

Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products

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ABSTRACT

Extracts from medicinal plants, many of which have been used for centuries, are increasingly tested in models of hepatotoxicity. One of the most popular models to evaluate the hepatoprotective potential of natural products is acetaminophen (APAP)-induced liver injury, although other hepatotoxicity models such as carbon tetrachloride, thioacetamide, ethanol and endotoxin are occasionally used. APAP overdose is a clinically relevant model of drug-induced liver injury. Critical mechanisms and signaling pathways, which trigger necrotic cell death and sterile inflammation, are discussed. Although there is increasing understanding of the pathophysiology of APAP-induced liver injury, the mechanism is complex and prone to misinterpretation, especially when unknown chemicals such as plant extracts are tested. This review discusses the fundamental aspects that need to be considered when using this model, such as selection of the animal species or *in vitro* system, timing and dose-responses of signaling events, metabolic activation and protein adduct formation, the role of lipid peroxidation and apoptotic versus necrotic cell death, and the impact of the ensuing sterile inflammatory response. The goal is to enable researchers to select the appropriate model and experimental conditions for testing of natural products that will yield clinically relevant results and allow valid interpretations of the pharmacological mechanisms.

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1. Introduction

Acetaminophen (APAP) is a safe and effective analgesic and antipyretic drug. However, an overdose can cause hepatotoxicity in experimental animals and humans. In fact, APAP hepatotoxicity is the most frequent cause of acute liver failure of any etiology in the western world, not only drug-induced liver failure (Larson et al., 2005; Larson, 2007). In addition, APAP has been used extensively during the last 40 years as a model toxicant, which allows investigation of drug-induced cell death *in vivo* and *in vitro*. Many basic concepts of drug toxicity were developed using this model. As a consequence, APAP toxicity is one of the most popular models to test potentially hepatoprotective agents, especially natural products. In addition, the fact that APAP overdose causes not only direct cell death but also triggers an innate immune response has increased the popularity of this model among immunologists (Adams et al., 2010; Jaeschke et al., 2012b). Unfortunately, too few researchers appreciate the complexity of the mechanisms of APAP-induced liver injury and misinterpretation of experimental results is common. As a result many highly conflicting data are being published. Although this may stimulate further research to refine the mechanism, when long-established fundamental principles of the model are ignored it not only wastes resources but prevents scientific progress. Therefore, the main objectives of this review are to summarize the current mechanistic understanding of APAP hepatotoxicity, and to discuss the different models being used and their relevance for the human pathophysiology. In addition, we point out pitfalls and common misconceptions of the mechanism and establish basic criteria that should be considered when using this or other models to test natural products.

2. Basic concepts in the mechanism of APAP toxicity

The intracellular signaling mechanisms of APAP-induced cell death were recently reviewed in great detail (Jaeschke et al., 2012a; Hinson et al., 2010; Jaeschke and Bajt, 2006). It is well established that the toxicity is initiated by the metabolism of a small fraction of the administered dose by P450 enzymes, mainly Cyp 2e1 and 1a2 (Zaher et al., 1998), to *N*-acetyl *p*-benzoquinone imine (NAPQI). This reactive metabolite is detoxified by glutathione (GSH) resulting in extensive hepatic GSH depletion (Mitchell et al., 1973; Knight et al., 2001). Concurrently, an increasing amount of NAPQI reacts with protein sulfhydryl groups, causing the covalent adduction of cellular proteins (Jollow et al., 1973; Cohen et al., 1997). Studies with the non-hepatotoxic regioisomer 3'-hydroxyacetanilide (AMAP) in mice indicated that the total protein binding in the cell is not as important as adducts in mitochondria (Tirmenstein and Nelson, 1989; Qiu et al., 2001). Mitochondrial protein binding triggers a mitochondrial oxidant stress (Jaeschke, 1990), which causes activation of apoptosis signal-regulating kinase 1 (Nakagawa et al., 2008) and c-jun N-terminal kinase (JNK) (Hanawa et al., 2008) and the amplification of the mitochondrial oxidant stress and peroxynitrite formation by mitochondrial JNK translocation (Saito et al., 2010a) (Fig. 1). The extensive oxidant stress finally triggers the opening of the membrane permeability transition (MPT) pore in the mitochondria with collapse of the membrane

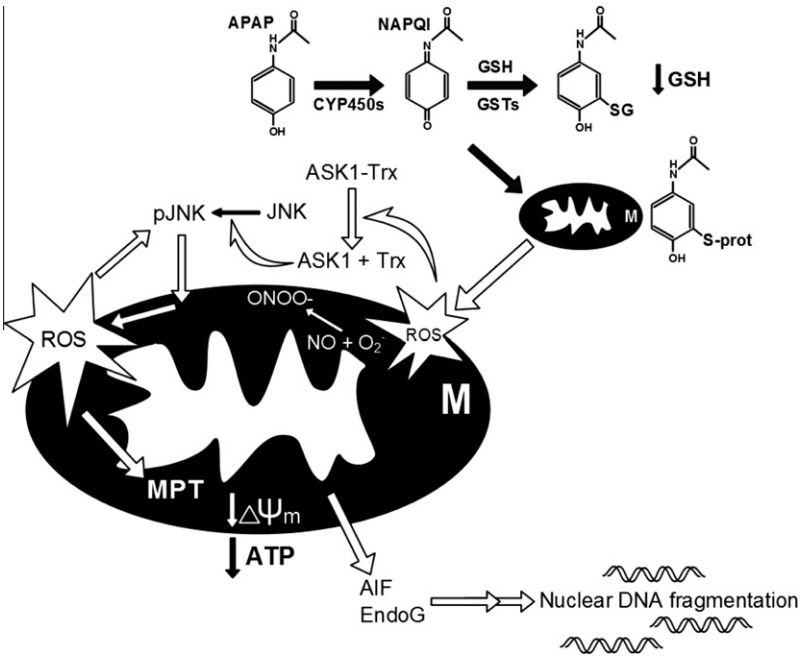

Fig. 1. Mechanisms of acetaminophen hepatotoxicity. Acetaminophen (APAP) is converted to the electrophile NAPQI through phase I drug metabolism. NAPQI can be detoxified by glutathione (GSH) or bind to proteins. Binding to mitochondrial proteins causes mitochondrial dysfunction and oxidative stress, which activates apoptosis signal-regulating kinase 1 (ASK1) and c-Jun N-terminal kinase (JNK). Translocation of JNK into mitochondria enhances the oxidative stress. The mitochondrial membrane permeability transition (MPT) occurs and the mitochondrial membrane potential is lost resulting in depletion of cellular ATP levels. The release of mitochondrial endonucleases (AIF and EndoG) leads to nuclear DNA fragmentation. The result of this sequence is cell necrosis.