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Acetaminophen Hepatotoxicity and Sterile Inflammation: the Mechanism of Protection of Chlorogenic Acid

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Abstract

Acetaminophen hepatotoxicity is characterized by extensive necrotic cell death and a sterile inflammatory response. A recent report suggested that a therapeutic intervention with chlorogenic acid, a dietary polyphenolic compound, protects against acetaminophen-induced liver injury by inhibiting the inflammatory injury. The purpose of this letter is to discuss a number of reasons why the protective mechanism of chlorogenic acid against acetaminophen hepatotoxicity does not involve an anti-inflammatory effect and provides an alternative explanation for the observed protection.

Keywords

Acetaminophen hepatotoxicity; sterile inflammation; neutrophils; natural products

I read with interest the recent manuscript by Zheng et al. (1) where the authors showed that treatment with chlorogenic acid (CGA) 1 h after an acetaminophen (APAP) overdose reduced liver injury. Since CGA attenuated hepatic MPO staining (indicator for neutrophil accumulation) and hepatic mRNA expression of various pro-inflammatory cytokines and chemokines, the authors concluded that the therapeutic use of CGA protected against APAP hepatotoxicity by preventing inflammatory injury mechanisms (1). There are several fundamental concerns with the experimental design of these studies and the mechanistic conclusions.

First, the authors treated mice with 400 mg/kg APAP for only 4 h. Thus, the assumption is that neutrophils caused most of the injury observed at that time (1). Neutrophil-induced liver injury has been studied extensively in models such as hepatic ischemia-reperfusion injury

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Conflict of Interest

The author declares no conflict of interest.

(2) and obstructive cholestasis (3). In these models, a neutrophil-mediated injury phase does not start before 6–10 h after the insult mainly due to the fact that neutrophils require not only activation and recruitment into the hepatic sinusoids but it also takes time to transcriptionally upregulate some of the required adhesion molecules and chemotactic factors for extravasation of neutrophils into the parenchyma, which is mandatory for any cytotoxicity of these inflammatory cells (4). Therefore, just based on these general mechanisms of neutrophil-induced liver injury, it is unlikely that CGA prevented an inflammatory injury.

Second, there is wide consensus in the literature that APAP hepatotoxicity triggers a sterile inflammatory response (5). This means that there has to be first severe necrotic cell death with release of damage-associated molecular patterns (DAMPs) in order to activate macrophages to generate pro-inflammatory mediators, which recruit neutrophils into the liver (5). The authors terminated the experiments at 4 h, i.e. at a time when the intracellular signaling mechanisms of reactive metabolite and protein adducts formation, mitochondrial dysfunction and DNA fragmentation are just beginning to cause the first cells to undergo necrosis (5). This means that APAP toxicity at 4 h is in the very early phase of sterile inflammation, i.e. cell necrosis and DAMPs release. Together, these data suggest that neither from the view of the general understanding of mechanisms of neutrophil-induced liver injury nor from the perspective of a sterile inflammatory response, is it possible that CGA protected because of an anti-inflammatory effect.

Third, there is direct evidence that neutrophils are unlikely to be involved in the injury mechanisms even at later time points despite their recruitment into the liver. Among others, blocking antibodies against CD18 and gene deficiency of CD18 and intercellular adhesion molecule-1, which are adhesion molecules critical for neutrophil cytotoxicity, and gene deficiency of gp91phox, which is part of the NADPH oxidase and leaves neutrophils without the capacity to generate reactive oxygen, had no effect on APAP toxicity (reviewed in: 6). Although some studies appear to support a role of neutrophils in the pathophysiology, many have been questioned due to methodological concerns with off-target effects (reviewed in: 6). Independently of any intervention, the fact that circulating or hepatic neutrophils are not activated or primed during the peak period of injury, e.g. 6 h after APAP in mice (7) and 24–48 h in humans (8), is direct evidence against neutrophil cytotoxicity after APAP overdose. In contrast, neutrophil activation can be detected during the recovery phase (8). Thus, the time course of neutrophil activation and direct interventions against neutrophils make it highly unlikely that these inflammatory cells contribute to the injury.

Fourth, the authors did not directly test the hypothesis that CGA protects against APAP toxicity by inhibiting the inflammatory response and neutrophil accumulation. They just showed a correlation between injury and different parameters of inflammation at one time point. However, this does not prove causality. If there is less injury due to other mechanisms, the same results would be obtained because the principal of sterile inflammation is that the inflammatory response depends on injury. Thus, at best the conclusions of the authors are not supported by the data presented.

In summary, as outlined there are a number of compelling reasons why the protective mechanism of CGA against APAP hepatotoxicity does not involve an anti-inflammatory

effect. How did CGA protect? Interestingly, CGA has been implicated as inhibitor of P450 enzymes (9). The administered dose of 400 mg/kg APAP requires at least 2–3 h to be metabolized (10). Since the authors treated the animals at 1 h after APAP, CGA had very likely an effect on reactive metabolite formation. If this was the case, this could explain all the results reported in this manuscript.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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