

Liuweiwuling tablets protect against acetaminophen hepatotoxicity: What is the protective mechanism?

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Abstract

Study of the effects of natural products, including

traditional Chinese Medicines, on acetaminophen hepatotoxicity has gained considerable popularity in recent years, and some of them showed positive results and even promising therapeutic potentials. A recent report suggested that Liuweiwuling tablets protect against acetaminophen hepatotoxicity and promote liver regeneration in a rodent model through alleviating the inflammatory response. However, several concerns exist regarding the limitations of the experimental design and interpretation of the data presented in this manuscript.

Key words: Acetaminophen hepatotoxicity; Liuweiwuling; Sterile inflammation; Natural products; Liver regeneration

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Core tip: The reduced inflammatory response and the increased liver regeneration by Liuweiwuling treatment are more likely secondary effects of the protection by inhibition of metabolic activation of acetaminophen rather than the primary mechanism of protection.

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TO THE EDITOR

We read with interest a recent article published by Lei *et al*^[1] in which the authors concluded that Liuweiwuling tablets alleviate acetaminophen (APAP) hepatotoxicity by inhibiting the inflammatory response in mice^[1]. The supporting evidence shown by the authors is the correlation between decreased levels of high-mobility group box 1 (HMGB1) protein and

inflammatory cytokines (TNF- α , IL-1 β) and the reduced liver injury after Liuweiwuling treatment. However, there are a number of concerns with the interpretation of the data and the mechanistic conclusions.

First, one of the main concerns is the relevance of the inflammatory response for APAP-induced liver injury. It is generally recognized that the initial necrotic cell death after APAP overdose results in the release of damage-associated molecular patterns (DAMPs), which bind to toll-like receptors on Kupffer cells and other inflammatory cells, and subsequently trigger the expression and release of pro-inflammatory cytokines^[2]. This leads to the activation and hepatic recruitment of innate immune cells^[2]. HMGB1 is one of such DAMPs that are released into the plasma after APAP overdose^[3]. However, its contribution to the injury mechanisms has been controversial. While in one study antibodies against HMGB1 were shown to dramatically reduce APAP toxicity^[4], other authors reported only a minor reduction in liver injury or even no effect^[5,6]. HMGB1 acts on Kupffer cells through TLR4 and induces expression of pro-inflammatory cytokines such as TNF- α and IL-1 β . The role of TNF- α in APAP toxicity has also been controversial. An early study suggested that TNF- α promotes APAP toxicity^[7]. However, other studies showed that mice deficient in TNF- α or TNF receptor-1 are not protected, and an increase in hepatic TNF- α concentration does not worsen the liver injury but might actually prime hepatocytes to facilitate the following liver regeneration^[8-10]. IL-1 β is also induced and released after TLR4 and Nalp3 inflammasome activation. However, the minor amount of IL-1 β generated during APAP hepatotoxicity has no impact on the injury process, and even 10000-fold higher IL-1 β levels cannot affect the liver injury^[11]. In addition, the fact that IL-1 receptor-deficient mice are not protected from the injury clearly indicates that IL-1 β signaling is not involved in the liver injury process^[11,12]. Immune cells, such as neutrophils and macrophages, are recruited into the liver and are being activated during or after the peak of the liver injury (≥ 12 h post-APAP in mice and ≥ 2 d in patients) by these pro-inflammatory mediators^[2,13]. However, extensive studies showed that immune cells are predominantly beneficial by removing cell debris and promoting liver regeneration rather than causing the liver injury^[2]. Also, it is very unlikely the late infiltrating cells could be responsible for the approximately 80% reduction of liver injury during the first 6 h post-APAP^[1]. In general, it takes at least 12 h before a neutrophil aggravates liver injury during a sterile inflammatory response^[14]. Taken together, there is no convincing evidence that these pro-inflammatory mediators or recruited innate immune cells could result in direct cell death after APAP hepatotoxicity, and the inflammatory response more likely promotes the recovery process rather than aggravates the initial liver injury^[2].

Second, it is well established that APAP hepatotoxicity

is initiated by the cytochrome P450-catalyzed formation of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI)^[15]. Therefore, any interference with this initial step would have profound effects on the intracellular signaling mechanisms of APAP-induced cell death and the following inflammatory response. Thus, it is essential to determine whether a natural product or any other compound has any effects on this initial step before other protective mechanisms can be proposed^[15,16]. Considering the facts that Liuweiwuling is given for 3 d before APAP at an extremely high dose (10.0 g/kg, 2 times/d by gavage) and the dramatic reduction of liver injury as early as 6 h when there is limited immune cell recruitment into the liver and no evidence that these cells are actually activated^[17], it is likely that Liuweiwuling tablets protect against the toxicity simply by inhibiting the metabolic activation of APAP. In fact, some of the components of Liuweiwuling tablets^[18], *e.g.*, varies schisandra lignans, have been shown to be effective inhibitors of Cyp 2E1 and Cyp1A2^[19], which are the critical enzymes for the metabolic activation of APAP^[15,16]. If the initial step of NAPQI formation is inhibited resulting in dramatically attenuated cell death, the reduced formation of inflammatory mediators is merely the consequence of the reduced early necrosis rather than the cause. Actually, inattention to this issue has led to misinterpretation of experimental results and mistaken conclusions in many APAP studies in recent years^[19-23].

The third concern comes from the effect of Liuweiwuling tablets on liver regeneration. It is known that severe liver injury after APAP overdose inhibits liver regeneration because of the active inhibition of cell cycle progression, while a more moderate liver injury triggers a prompt and vigorous regeneration response^[24,25]. Since the liver injury was largely reduced after Liuweiwuling treatment^[1], it is possible that the increased liver regeneration in the Liuweiwuling-treated mice is a consequence of the reduced liver injury and does not reflect a direct effect of the intervention on liver regeneration process. To better support the argument that Liuweiwuling directly promotes liver regeneration after APAP overdose, any inference with the injury process needs to be avoided and treatment needs to be started after the peak of injury.

In summary, although the protective effect of Liuweiwuling tablets against APAP hepatotoxicity is interesting and was convincingly demonstrated, the proposed protective mechanism is questionable. The reduced inflammatory response and the increased liver regeneration by Liuweiwuling treatment are more likely secondary effects of the protection by inhibition of metabolic activation of APAP rather than the primary mechanism of protection. Studies with natural products need to be carefully designed and consider all aspects of the mechanism of APAP-induced cell death in order to obtain relevant information on the therapeutic potential and mechanism of action of the product.

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