Acetaminophen Overdose-induced Liver Injury in Mice Is Mediated by Peroxynitrite Independently of the Cyclophilin D-regulated Permeability Transition

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Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is safe at therapeutic doses. However, ~~However, when administered overdose,~~ APAP overdose can cause liver damage in humans and mice. Despite extensive research ~~for~~ over several decades, the underlying molecular mechanisms of hepatocyte injury are ~~still~~ not ~~fully~~ understood~~.~~, Thus,~~limiting~~ the development and therapeutic application of novel cytoprotective agents in APAP-induced liver injury have been limited ~~(Jaeschke 2006 & Saito 2010)~~ .[1,2] ~~What has become clear is that~~Mitochondria play a key role in both early-~~-~~stage~~s of~~ cellular injury (interaction between~~s of~~ thiol-intermediate reagent~~,~~ N-acetyl-p-benzoquinone imine [~~,~~NAPQI]~~,~~ ~~with~~ glutathione, and proteins~~[,]~~ as well as ~~accompanied by~~ antioxidant~~s~~ and nitrative stress) and~~And~~ subsequent phase propagation (signaling followed by hepatocellular death) .[3,4] ~~mitochondria appear to play a key role~~ ~~(Cover 2005 & Hanawa 2008). Evidence has been shown,~~ ~~a~~After in vitro or in vivo hepatocyte exposure ~~of hepatocytes~~ to APAP ~~in vitro or in vivo~~, mitochondria readily~~easily~~ undergo permeabilization of the outer membrane with subsequent~~, thus inducing~~ necrosis~~tic cell death, secondary to largely through~~ caspase-independent mechanisms. How ~~exactly~~ NAPQI and ~~its~~ subsequent signaling events lead to mitochondrial permeabilization ~~at present~~ is un~~not~~ known. It has been suggested that ~~the process may involve~~ the ~~transition~~ process of mitochondrial permeability (mPT) may be involved. The ~~The~~ mPT refers to the ~~is a functional term that involves~~ sustained opening of a megapore ~~that~~ involving~~encompasses~~ both internal and external mitochondrial membrane~~s, which~~, allow~~sing the~~ exchange of solutes smaller than< 1.5 kDa~~, leading~~ to cause mitochondrial swelling, external membrane rupture, and ~~release of~~ proapoptotic protein~~s~~ release~~-~~. Although the physiologic~~al~~ properties of mPT are ~~have been~~ well-described ~~studied~~, the molecular nature of this pore remains poorly defined. Originally, the ADP-/-ATP translocator (ANT) and the voltage-~~-~~dependent anionic transporter (VDAC) were~~have been~~ attributed a crucial role in this process. However, recent work shows that ~~, but this concept had to be reviewed recently, it was found that the~~ mitochondria of ANT or VDAC knockout mice a~~w~~ere still susceptible to mPT effects~~Capable of being subjected to.~~

~~On the other hand, the c~~Cytophilin D (CypD) matrix protein appears to be an important factor ~~critical actor involved~~ in mPT pore~~the~~ regulation ~~of the mPT pore~~. Studies of m~~M~~itochondria~~l studies~~ isolated from mice with a genetic deletion of CypD ~~have clearly~~ demonstrate~~d that these mitochondria were much more~~higher

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(but not full) resistancet to mPT inducers compared than to wild-type mitochondria (though not fully protected). As an alternative to the genetic deletion of CypD, tThe interaction betweenof CypD andwith the mPT pore can also be disrupted by pharmacological inhibition with, eg with cyclosporin A (CsA) or other specific cyclophilin ligands. Thuserefore, the demonstration of protective effects provided by CsA against drug toxicitythe effects of toxic drugs haves been widely reported as supporting the role of used to make an argument for mPT involvement.

Consistent withBased on this concept of cytostatic CsA, severala number of independent studies have implicatedprovided experimental evidence that mPT could indeed be implicated in APAP-induced liver toxicity. However, one caveat is that administration of CsA, given at high doses (as used in some of the mouse studies), may inhibit drug transporters in the domain of the canalicular membrane domain and also induce cholestasis. This process maycould alter the kinetics of APAP and / or its metabolites. ImportantlyIn addition, and importantly, CsA not only binds not onlyto mitochondrial CypD, but also to other forms of cyclophilin (eg,including cytosolic CypA). The CypA-/-CsA complex is subsequently linked to calcineurin, a Ca^{2+}-/-calmodulin-activated serine-/-threonine phosphatase that has is been mechanically involved in the immunosuppressive effects of CsA. LastlyFinally, CsA has been shown to exert other calcineurin-independent effects on c-jun NH-2--terminusal terminal kinase (JNK) signaling. Therefore, the role of CypD-dependent mPT in the APAP hepatotoxicity should be reviewed, based solely on the protective effects provided by CsA, should be reviewed. In fact, sStudies in isolated hepatocytes have shownprovided evidence that, with increasing time and cellular stress, CsA eventually loses its protective effects towards APAP-induced cellular injury. However, it is unnot known whether this change also occurs in vivo., Also, and, more importantly, tthe mechanism by which APAP toxicity is "insensitivee to CsA" treatment is not understoodof APAP toxicity has remained enigmatic.

The aim of this study was to investigate whether APAP inducesexerts mitochondrial permeabilization through mPT and / or through other mechanisms CypD-independent mechanisms. of CypD, Busing both the in vivo pharmacological inhibitionors of CypD and a genetic approach usingwith deficient CypD-deficient (Ppif -/-) mMice were used in this study. The dataResults suggest that high APAP doses of APAP induce mitochondrial peroxynitrite stress, which that directly triggers mitochondrial permeabilization without the CypD involvement of CypD.

Results

Pharmacological inhibition or genetic depletion of mitochondrial CypD does not protect against the APAP-induced hepatotoxicity. of APAP

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To investigate the mechanistic role of CypD-controlled mPT compared toversus other modes of cell death in APAP-induced liver injury, a previously characterized mouse model was used. 20 Acetaminophen (600 mg/day) was administered intraperitoneally given to 20 wild-type male micees (Ppif$^{+/+}$ Kg, ip). As expected, APAP caused typical centrilobular necrosis, which was evident at 8 h post-dose and became more severe at 24 h. The development of necrosis , paralleled to the highly increased plasma ALT activity of plasma ALT (Fig 1A, B, D). Because the choice of solvent may have significantly aeffects on APAP bioactivation and / or subsequent recruitment of immune cells recruitment and thus on the extent of liver injury, we first determined the effects of Solutol HS 15 for , Para Pparenteral administration of lipophilic compounds to, and compared with those of hot saline solution used to dissolve APAP. Unlike dimethylsulfoxide, It was found that Solutol HS 15 did not affect, in contrast to dimethylsulfoxide, had no apparent effects on plasma ALT activity (Table 1). Therefore, Solutol HS 15 was used as a the vehicle for all subsequent experiments.

Previous reports from various laboratories have shown that CsA can effectively protects mouse hepatocytes from APAP-induced injury both in vitro and in vivo. However, CsA may producehave a number of several off-target effects, including those unnot related to CypD. To avoid these confounding factors, the CsA analog, Debio 025 was used. Debio 025 is, which is a more selective CypD mitochondrial inhibitor with a 3,000-fold smallerand whose potency compared to CsA in to inhibiting the immune system (via the calcineurin-mediated pathways) is> 3,000 times less than The CsA. Debio 025 (10 mg/kg, ip) was administered intraperitoneally 1.5 h after APAP administration (time at whichwhen APAP bioactivation was largely completed and most of the hepatic GSH had already been consumed by NAPQI) was injected, to thus minimizeing drug drug interactions. Surprisingly, it was found that Debio 025 did not protect againstfrom APAP induced hepatotoxicity (Fig. 1C, D). A pilot study showedrevealed that there was a similar lack of protectionve effects when administering Debio 025 was administered simultaneously with APAP (data not shown), indicating that the lack of protection was not related simply due to late administration of the CypD inhibitor. These findings suggest that, in addition to the CypD dependent mode of action of mPT, there may be another mode of mitochondrial permeabilization induced by high doses of APAP.

To corroborate these findings and to totally exclude theany possibilityle of drug interactions due to the presence of the pharmacologicaal inhibitors, we then determined the extent of APAP-induced liver injury in a mouse genetic model of CypD depletion (Ppif mice;) (Figure 2A). We first had to confirmedcheck that these CypD-deficient mice exhibited similar rates of APAP bioactivation as to their wild-type controls. To do soTherefore, hepatic GSH consumption was measured for 90 min followingafter administration of a hepatotoxic dose of APAP in Ppif$^{-/-}$ mice and their wild-type littermates for the first 90 min (a marker established for the extension of NAPQI formation). Although Ppif$^{-/-}$ mice initially had initially 30% higher GSH levels (+ 30%), no significant differences were found in the extent of

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GSH depletion between the two genotypes (Figure 2B). We nextthen evaluated the degree of liver injury after 4, 8, and 24 h in both Ppif$^{+/+}$ and Ppif$^{-/-}$ mice injected with 600 mg intraperitoneal APAP (600 mg / kg, ip). Consistent In line with the results of the Debio 025 resultsexperiments, the Ppif$^{-/-}$ mice were not protected from APAP toxicity at this high dose, but developed typical centrilobular necrosis after 24 h that resembled that of , whose expression was not different from that of the wWild-type animals, (Fig. 2D). Taken together, these data indicate that mitochondrial signaling involved in APAP hepatotoxicity includes an independent mode of CypD , at least at this high doses. In Howevercon, trast, at much lower doses, inhibition of the CypD pathway may still allow cytoprotection at much lower APAP doses, as shown in a recent report.

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