Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. While safe at therapeutic doses, however, when administered overdose, overdoses of APAP can cause liver damage in humans and mice. Despite extensive research for several decades, the underlying molecular mechanisms of hepatocyte injury are still not fully understood, limiting the development and therapeutic application of novel cytoprotective agents against APAP-induced liver injury (Jaeschke & Bajt 2006; Saito et al. 2010). What has become clear is that mitochondria play a key role in both the early stages of cell injury (interactions of the thiol-intermediate reagent, N-acetyl-p-benzoquinone imine [NAPQI], with glutathione and proteins, accompanied by antioxidants and nitrative stress) and the subsequent phase propagation (signaling followed by hepatocellular death). Mitochondria appear to play a key role (Cover et al. 2005; Hanawa et al. 2008). Evidence has shown that after exposure of hepatocytes to APAP in vitro or in vivo, facilitates mitochondrial permeabilization of the mitochondrial outer membrane, occurs easily, thus inducing necrotic cell death, largely-primarily through caspase-independent mechanisms. How exactly NAPQI and its subsequent signaling events lead to mitochondrial permeabilization at present is not currently unknown at present. It has been suggested that the process may involve the transition of mitochondrial permeability (mitochondrial permeability transition or mPT). The mPT is a functional term that involves the sustained opening of megapore that encompasses across both the internal inner and external outer mitochondrial membranes. This allows the exchange of solutes of <1.5 kDa, leading to mitochondrial swelling, external membrane rupture, and release of proapoptotic proteins. Although the...
physiological properties of the mPT pore have been well extensively studied, the its molecular nature of this pore remains poorly defined. Originally, the ADP/ATP translocator (ANT) and the voltage-dependent anionic transporter (VDAC) have been were initially attributed crucial roles, but these have recently been drawn into question a crucial role, but this concept had to be bellow recently reviewed disputed recently, it was found that because of the occurrence of permeability transition could still occur in the mitochondria of ANT or VDAC knockout mice were still Capable of being subjected to. On the other hand, Genetic genetic studies support a major role for cyclophilin D (CypD) matrix protein appears to be a critical actor involved in the regulation of the mPT pore. original Genetic genetic studies isolated from mice with a genetic deletion of CypD have clearly demonstrated that these mitochondria were much more resistant increased resistance to mPT inducers than the compared to wild-type mitochondria (though although they were not fully protected). As an alternative to the genetic deletion of CypD, pharmacological inhibition — e.g., for instance using cyclosporin A (CsA) or other specific cyclophilin ligands — can also disrupt the interaction of CypD with the mPT pore can also be disrupted by pharmacological inhibition, eg with cyclosporin A (CsA) or other specific cyclophilin ligands. Therefore, the demonstration of protective effects provided demonstrated by CsA against the effects of toxic drugs toxicity has have been widely been widely used to make an the argument for support mPT the involvement of mPT.

Based on this concept of cytostatic effects of CsA, a number of independent studies have have provided experimental evidence that mPT could is indeed be implicated in APAP-induced liver toxicity. However, one caveat is that 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 could alter the kinetics of APAP and/or and/or its metabolites. In addition, and importantly, CsA not only binds not only to mitochondrial CypD but also to other forms of cyclophilin, including cytosolic CypA. The CypA/CsA CypA/CsA complex is subsequently linked to calcineurin, a Ca2+/calmodulin activated serine/threonine phosphatase that has been mechanically involved in the immunosuppressive effects of CsA. Finally, CsA has also been shown to exert other calcineurin-independent effects on NH2-terminal kinase (JNK) signaling. Therefore, the role
of CypD-dependent mPT in APAP hepatotoxicity, based solely on the protective effects provided by CsA, should be reviewed. Notably, studies in isolated hepatocytes have provided evidence that with increasing time and cell stress, CsA eventually loses its protective effects against APAP-induced cell injury with increasing time and cell stress. However, it is not known whether this occurs in vivo.

Additionally, and more importantly, the mechanism of "insensitive to CsA insensitivity" of APAP toxicity has remained obscure.

The aim of this study was to investigate whether APAP exerts caused mitochondrial permeabilization, either through mPT and/or through other mechanisms, independently of CypD, using We used both in vivo pharmacological inhibitors of CypD and a genetic approach with deficient-CypD-deficient (Ppif−/−) mice. The data suggest that high doses of APAP induce mitochondrial peroxynitrite stress that directly triggers mitochondrial permeabilization without the involvement of CypD.

Results

Pharmacological inhibition or genetic depletion of mitochondrial CypD does not protect against the APAP hepatotoxicity of APAP. A previously characterized mouse model was used to investigate the mechanistic role of CypD-controlled mPT versus other modes of cell death in APAP-induced liver injury. Twenty Acetaminophen acetaminophen APAP (600 mg./day) was given to male wild-type male mice (Ppif−/−). As expected, APAP caused typical centrilobular necrosis, which was evident at 8 h post-dose and became more severe at 24 h, paralleling the highly increased activity of plasma ALT. Because the choice of solvent may have an effect on APAP bioactivation and/or the subsequent recruitment of immune cells and thus the extent of liver injury, we first determined the effects of Solutol HS-15, used in Parenteral administration of lipophilic compounds, and compared it with those of the hot saline solution used to dissolve APAP. It was found that Solutol HS-15 in contrast to
Solutol HS-15 had no apparent effects on plasma ALT activity (Table 1). Therefore, Solutol HS-15 was used as the vehicle for excipient in all subsequent experiments.

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

To corroborate these findings and to totally exclude any possible drug interactions due to the presence of the pharmacological inhibitors, we determined the extent of APAP-induced liver injury in a CypD-depletion mouse genetic model of CypD-depletion (Ppif−/−) mice (Figure 2A). We first had to check confirmed the APAP bioactivation rates of these CypD-deficient mice exhibited similar rates of APAP bioactivation were had similar APAP bioactivation rates to those of as their the wild-type controls. Therefore Specifically, hepatic GSH consumption (a marker established for the extension of NAPQI formation) was measured for 90
min after-following administering administration of a hepatotoxic dose of APAP in to Ppif−/− mice and their wild-type littermates for the first 90 min (a marker established for the extension of NAPQI formation). Although Ppif−/− mice had initially had higher GSH levels (+30%), no significant differences were found in the extent level of GSH depletion between the two genotypes (Figure Fig. 2B). We then evaluated the degree of liver injury after 4, 8, and 24 h in both Ppif−/− and Ppif−/− mice injected with APAP (600 mg/kg, ip). In line Consistent with the results of the Debio 025 experiments, the Ppif−/− mice were not protected from APAP toxicity at this high dose, but developed typical centrilobular necrosis after 24 h, after 24 h, whose similar in expression was not different from to that of in the Wild wild-type animals, after 24 h (Fig. 2D). Taken together Overall, these data indicate that at the high dose used here, the mitochondrial signaling involved in APAP hepatotoxicity includes an a mode CypD independent mode of CypD, at least at this high dose. In contrast, a recent report suggests that the at much lower doses, inhibition of the CypD pathway may still allows cytoprotection when lower doses are administered, as shown in a recent report.

