Letter to the Editor Regarding the Article "Rotenone Increases Isoniazid Toxicity but Does Not Cause Significant Liver Injury: Implications for the Hypothesis that Inhibition of the Mitochondrial Electron Transport Chain Is a Common Mechanism of Idiosyncratic Drug-Induced Liver Injury" by Cho and Co-Workers, 2019

Bernard Fromenty
INSERM, Université de Rennes, INRAE, Nutrition, Metabolisms, and Cancer (NuMeCan) Institut, UMR_A 1341, UMR_S 1241 , F-35000 Rennes , France.

To the Editor: Idiosyncratic drug-induced liver injury (iDILI) is a major issue for the treated patients because of its unpredictability and its potential severity. It is also a concern for the pharmaceutical companies since severe or lethal liver injury can lead to the withdrawal of drugs from the market, or earlier during clinical trials, thus causing significant financial losses.[1] While immune response plays a major role,[2] it is now accepted that mitochondrial dysfunction is also an important mechanism whereby drugs can induce iDILI.[1,3,4] The unpredictability of drug-induced mitochondrial dysfunction in iDILI can be due to different causes such as drug-drug pharmacokinetic and/or pharmacodynamic interactions, the presence of an underlying liver disease, or different types of genetic predisposition affecting mitochondrial function.[1,5] Genetic defects affecting the mitochondrial electron transport chain (mtETC) or the mitochondrial fatty acid oxidation (mtFAO) pathway have been reported to favor mitochondrial dysfunction and liver injury induced by several drugs such as the anticonvulsant drug valproic acid and some antiretroviral agents.[1,5]

In a recent article published in *Chemical Research in Toxicology*, Cho and co-workers treated C57BL/6 mice with rotenone (a prototypical inhibitor of the MRC complex I), isoniazid (an antituberculosis drug inhibiting the MRC complex II), or both compounds, over a six-week period.[6] Whereas treatment with rotenone alone (0.05 or 0.1% w/w in food) or isoniazid alone (0.2% w/w in food) did not cause death among the animals, the coadministration of rotenone and isoniazid led to lethality in 100% of the mice. This toxicity was not related to liver injury as assessed by hepatic histology and serum glutamate dehydrogenase (GLDH) activity. Notably, the latter investigations were performed after only 3 and 6 days since the cotreated mice did not survive afterwards. From these results, the authors conclude that inhibition of the mtETC is not a significant mechanism of iDILI.[6] Although this *in vivo* study provides interesting data, this conclusion is somewhat misleading for the following reasons.

First and foremost, there is already strong evidence that mtETC impairment is responsible for liver injury with different drugs, especially with drugs altering the replication or translation of mitochondrial DNA (mtDNA), which encodes for 13 mtETC polypeptides. Of note, such

alteration eventually leads to an impairment of electron transfer from both complexes I and II and onwards of the hepatic mtETC. For instance, phase II clinical trials with the anti-HBV drug fialuridine, which inhibits mtDNA replication,[7] were prematurely interrupted due to serious adverse effects including unmanageable lactic acidosis, microvesicular steatosis and liver failure requiring liver transplantation, or even leading to death.[8,9] Similar adverse effects can be induced by different antiretroviral nucleoside reverse-transcriptase inhibitors (NRTIs) such as zalcitabine (ddC), stavudine (d4T) and didanosine (ddI),[10,11] which also strongly inhibit mtDNA replication.[7,10] Experimental investigations with fialuridine and NRTIs demonstrated the major role of mtDNA depletion and mtETC impairment in the development of liver injury.[10,12,13] NRTI-induced hepatotoxicity occurs only in some patients, thus suggesting the role of underlying factors such as genetic predisposition.[5,14] Furthermore, other experimental investigations support the role of mtETC impairment in liver injury induced by amiodarone,[15,16] perhexiline[17] and buprenorphine.[18]

Second, in the study of Cho and colleagues, the premature death from extra-hepatic causes of the mice cotreated with rotenone and isoniazid most probably precluded the possibility to observe delayed mitochondrial toxicity in liver and subsequent hepatic injury.[6] Notably, liver is not the primary target organ during rotenone toxicity.[19] Human and animals studies showed that rotenone-induced mitochondrial toxicity causes respiratory depression, cardiovascular collapse and severe metabolic (i.e. lactic) acidosis.[19] In addition to heart and lungs, kidney and spleen are also damaged after rotenone exposure.[20] Regarding isoniazid, although hepatotoxicity is a significant adverse event in treated patients, hypotension, renal failure and metabolic acidosis could also occur.[21,22] A thorough necropsy of the deceased mice might have uncovered which vital organs were severely damaged by rotenone. Moreover, *in vitro* investigations in hepatocytes might have permitted to evaluate the toxicity of rotenone, isoniazid and their combination. Indeed, previous investigations reported that rotenone and isoniazid could induce toxicity in hepatic cells.[23,24]

Third, the authors looked for hepatic injury in liver sections by using hematoxylin and eosin staining. However, this staining method is not appropriate in order to detect microvesicular steatosis,[25] a liver lesion commonly occurring with drugs and toxins inducing mitochondrial dysfunction.[7,26] Regardless of the pathophysiological context, minor or moderate microvesicular steatosis is better detected with Oil red O or Sudan III staining on frozen liver section.[25,27]

Investigations in animals are useful in order to study DILI including iDILI, but different factors may greatly modulate the severity of liver injury such as drug distribution and metabolism as well as animal species and strain.[1,28,29] Interestingly, isoniazid did not induce microvesicular steatosis in C57BL/6J mice, whereas this liver lesion was observed in other strains such as BALB/cJ, DBA/2J and LG/J mice.[30] The latter study and others[1,14,31,32] underline the importance of genetic susceptibility in the occurrence of drug-induced liver injury, in particular when considering genes encoding for mitochondrial proteins. Importantly, drug-induced mtETC toxicity can cause not only microvesicular steatosis and cell death but also other liver lesions such as steatohepatitis and cholestasis.[5,17,33] Hence, more investigations are clearly needed in order to identify the main factors able to modulate drug-induced mitochondrial dysfunction in liver. While *in vivo* investigations may better reflect the complexity of iDILI, complementary *in vitro* experiments are also useful to decipher the mechanisms whereby drugs can be toxic for mitochondria.

Bernard Fromenty

INSERM, Univ Rennes, INRA, Institut NUMECAN (Nutrition Metabolisms and Cancer)

UMR_A 1341, UMR_S 1241, F-35000 Rennes, France

AUTHOR INFORMATION

ORCID

Bernard Fromenty: 0000-0003-2994-5465

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