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**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**

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► **To cite this version:**

Bernard Fromenty. 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. *Chemical Research in Toxicology*, 2020, 33 (1), pp.2-4. 10.1021/acs.chemrestox.9b00416 . hal-02442529

**HAL Id: hal-02442529**

**<https://univ-rennes.hal.science/hal-02442529>**

Submitted on 12 Feb 2020

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1 Letter to the Editor Regarding the Article "Rotenone Increases Isoniazid Toxicity but Does Not  
2 Cause Significant Liver Injury: Implications for the Hypothesis that Inhibition of the  
3 Mitochondrial Electron Transport Chain Is a Common Mechanism of Idiosyncratic Drug-  
4 Induced Liver Injury" by Cho and Co-Workers, 2019  
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13 To the Editor: Idiosyncratic drug-induced liver injury (iDILI) is a major issue for the treated  
14 patients because of its unpredictability and its potential severity. It is also a concern for the  
15 pharmaceutical companies since severe or lethal liver injury can lead to the withdrawal of drugs  
16 from the market, or earlier during clinical trials, thus causing significant financial losses.<sup>1</sup> While  
17 immune response plays a major role,<sup>2</sup> it is now accepted that mitochondrial dysfunction is also  
18 an important mechanism whereby drugs can induce iDILI.<sup>1,3,4</sup> The unpredictability of drug-  
19 induced mitochondrial dysfunction in iDILI can be due to different causes such as drug-drug  
20 pharmacokinetic and/or pharmacodynamic interactions, the presence of an underlying liver  
21 disease, or different types of genetic predisposition affecting mitochondrial function.<sup>1,5</sup> Genetic  
22 defects affecting the mitochondrial electron transport chain (mtETC) or the mitochondrial fatty  
23 acid oxidation (mtFAO) pathway have been reported to favor mitochondrial dysfunction and  
24 liver injury induced by several drugs such as the anticonvulsant drug valproic acid and some  
25 antiretroviral agents.<sup>1,5</sup>  
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36 In a recent article published in *Chemical Research in Toxicology*, Cho and co-workers  
37 treated C57BL/6 mice with rotenone (a prototypical inhibitor of the MRC complex I), isoniazid  
38 (an antituberculosis drug inhibiting the MRC complex II), or both compounds, over a six-week  
39 period.<sup>6</sup> Whereas treatment with rotenone alone (0.05 or 0.1% w/w in food) or isoniazid alone  
40 (0.2% w/w in food) did not cause death among the animals, the coadministration of rotenone  
41 and isoniazid led to lethality in 100% of the mice. This toxicity was not related to liver injury  
42 as assessed by hepatic histology and serum glutamate dehydrogenase (GLDH) activity.  
43 Notably, the latter investigations were performed after only 3 and 6 days since the cotreated  
44 mice did not survive afterwards. From these results, the authors conclude that inhibition of the  
45 mtETC is not a significant mechanism of iDILI.<sup>6</sup> Although this *in vivo* study provides  
46 interesting data, this conclusion is somewhat misleading for the following reasons.  
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56 First and foremost, there is already strong evidence that mtETC impairment is responsible  
57 for liver injury with different drugs, especially with drugs altering the replication or translation  
58 of mitochondrial DNA (mtDNA), which encodes for 13 mtETC polypeptides. Of note, such  
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3 alteration eventually leads to an impairment of electron transfer from both complexes I and II  
4 and onwards of the hepatic mtETC. For instance, phase II clinical trials with the anti-HBV drug  
5 fialuridine, which inhibits mtDNA replication,<sup>7</sup> were prematurely interrupted due to serious  
6 adverse effects including unmanageable lactic acidosis, microvesicular steatosis and liver  
7 failure requiring liver transplantation, or even leading to death.<sup>8,9</sup> Similar adverse effects can  
8 be induced by different antiretroviral nucleoside reverse-transcriptase inhibitors (NRTIs) such  
9 as zalcitabine (ddC), stavudine (d4T) and didanosine (ddI),<sup>10,11</sup> which also strongly inhibit  
10 mtDNA replication.<sup>7,10</sup> Experimental investigations with fialuridine and NRTIs demonstrated  
11 the major role of mtDNA depletion and mtETC impairment in the development of liver  
12 injury.<sup>10,12,13</sup> NRTI-induced hepatotoxicity occurs only in some patients, thus suggesting the  
13 role of underlying factors such as genetic predisposition.<sup>5,14</sup> Furthermore, other experimental  
14 investigations support the role of mtETC impairment in liver injury induced by amiodarone,<sup>15,16</sup>  
15 perhexiline<sup>17</sup> and buprenorphine.<sup>18</sup>

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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.<sup>6</sup> Notably, liver is not the primary target organ during rotenone toxicity.<sup>19</sup> Human and animals studies showed that rotenone-induced mitochondrial toxicity causes respiratory depression, cardiovascular collapse and severe metabolic (i.e. lactic) acidosis.<sup>19</sup> In addition to heart and lungs, kidney and spleen are also damaged after rotenone exposure.<sup>20</sup> Regarding isoniazid, although hepatotoxicity is a significant adverse event in treated patients, hypotension, renal failure and metabolic acidosis could also occur.<sup>21,22</sup> 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.<sup>23,24</sup>

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,<sup>25</sup> a liver lesion commonly occurring with drugs and toxins inducing mitochondrial dysfunction.<sup>7,26</sup> 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.<sup>25,27</sup>

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

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54 I am very grateful to Dr Julie Massart for her critical reading of this letter.  
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